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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:)
)
PETITION OF GENERAL MOTORS) R93-13
CORPORATION TO AMEND)
35 ILL. ADMIN. CODE 303.322)
(Site-Specific Regulation for Fluoride))

NOTICE OF FILING

To: Mary Gade, Director
Illinois Environmental
Protection Agency
P.O. Box 19276
2200 Churchill Road
Springfield, Illinois 62794

Bruce Carlson
Office of General Counsel
Illinois Environmental
Protection Agency
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Springfield, Illinois 62794

Bill Denham
Department of Energy & Natural Resources
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Springfield, Illinois 62704-1892

PLEASE TAKE NOTICE that today I have filed with the Office of the Clerk of the Illinois Pollution Control Board, **Motion to Waive Requirement to Submit 200 Signatures and Petition to Amend Site-Specific Regulation**, copies of which are herewith served upon you.

GENERAL MOTORS CORPORATION,
POWERTRAIN DIVISION

By: _____
One of its Attorneys

Dated: June 23, 1993

ROSS & HARDIES
Raymond T. Murphy
David L. Rieser
150 North Michigan Avenue
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(312) 558-1000

THIS FILING SUBMITTED ON RECYCLED PAPER

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:)
) R93- 13
PETITION OF GENERAL MOTORS)
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MOTION TO WAIVE REQUIREMENT TO SUBMIT 200 SIGNATURES

Now comes GENERAL MOTORS CORPORATION, POWERTRAIN DIVISION, by and through its attorneys, ROSS & HARDIES and requests the Pollution Control Board to waive the requirement to submit 200 signatures with its Petition to Amend Regulations and states in support as follows:

1. General Motors operates an iron foundry in Danville, Illinois which employs more than 11,000 persons and annually contributes more than $80 million to the area economy. The facility produces more than 800 tons per day of iron castings for General Motors.

2. This motion is attached to a Petition to amend the current site specific regulation for this facility. This petition requests the Board to modify the fluoride water quality originally granted to GM.

3. The Board has waived signature requirements for site specific rule change petitions in the past, most recently with regard to a rule change petition docketed as R93-8 by order dated February 4, 1993.

4. Granting this motion is in the public interest in light of the products produced by the facility and the benefits of the facility's operation.

WHEREFORE, GENERAL MOTORS respectfully requests the Board to waive the requirement to submit 200 signatures in support of its Site Specific Rule Change Petition.

Respectfully Submitted,

GENERAL MOTORS CORPORATION,
POWERTRAIN DIVISION

By: 
One of its Attorneys

ROSS & HARDIES
Raymond T. Murphy
David L. Rieser
150 North Michigan Avenue
Chicago, Illinois 60601
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PETITION TO AMEND SITE-SPECIFIC REGULATION

GENERAL MOTORS CORPORATION, POWERTRAIN DIVISION (GM), by and through its attorneys, ROSS & HARDIES, petitions the Illinois Pollution Control Board pursuant to 415 ILCS 5/27 and 35 Ill. Admin. Code 102 Subpart C to amend 35 Ill. Admin. Code 303.322 (Site Specific fluoride standard) and states in support as follows:

PROPOSED SITE-SPECIFIC REGULATION

1. GM is seeking to modify 35 Ill. Admin. Code 303.322 which established a site-specific water quality standard for fluoride in an unnamed tributary of the Vermilion River and the Vermilion River from the juncture of the unnamed tributary to the Indiana border. This site-specific water quality standard was intended to provide relief for fluoride discharges from GM's Danville plant. The Board adopted this regulation under docket number R78-7 on September 24, 1981. GM seeks to increase the fluoride water quality standard for the unnamed tributary and the

Vermilion River from the juncture of the unnamed tributary to a point 0.9 miles downstream of that juncture from 5 mg/l to 10 mg/l. By virtue of this request, the water quality standard for the stretch of the Vermillion River to the Indiana border previously covered by this relief would revert to the General Use Standard of 1.4 mg/l. The Agency has reviewed this Petition prior to filing it with the Board and has indicated that it has no objection to the relief.

The revised standard would read as follows:

Section 303.322 Unnamed Tributary of the Vermilion River

The fluoride standard of Sec. 302.208 shall not apply to waters of the State which are located from the point of a discharge to an unnamed tributary of the Vermilion River, said point being located 3900 feet south of the Vermilion River, 1900 feet north of I-74, at 40°6'35" north latitude and 87°68'52" west longitude, to the confluence of said unnamed tributary with the Vermilion River and from there downstream to ~~its juncture with the Indiana state border~~ a point 0.9 river miles downstream of the juncture at the crossing of a Norfolk and Western Railroad Bridge. Fluoride levels in such waters shall meet a water quality standard for fluoride (Storet Number 009050) of ~~5~~ 10 mg/l.

STATEMENT OF REASONS

Description of the Facility

2. GM's Danville Plant is an iron foundry which employs more than 1000 persons and annually contributes more than

$80 million to the Danville area economy. The foundry is located in a rural industrial area on the border between Danville and the Village of Tilton, immediately north of Interstate 74. The foundry manufactures ductile and grey iron castings for the automotive industry. The unit processes include cupola melting, sand molding, rough finishing and annealing of castings that are used in the production of intake manifolds, transmission parts and brake parts. The facility produces approximately 800 tons per day of iron castings.

3. The facility draws its make-up water for process and cooling purposes from the Vermilion River. The water is used for a variety of purposes including cupola shell cooling, cupola emission cleaning, slag sluicing, non-contact machine cooling and dust collecting.

4. The facility discharges 400,000 to 750,000 gpd of waste water including both process waste water and non-contact cooling water. Process waste water, non-contact cooling water and storm water receive treatment prior to discharge through Outfall 002. The treatment process includes: primary settling of process waste waters, chemical treatment and mixing, settling in a 12 million gallon settling basin, filtering through multimedia pressure filters and cooling as required through a cooling tower. The outfall discharges to a ditch which joins the Vermilion River 1400 feet from the outfall.

Description of the Present Inability to Achieve Compliance

5. GM described its inability to comply with the general use fluoride standard in great detail in R78-7. In that proceeding, GM experts testified that the facility exceeded the fluoride water quality standard due to the elevated levels of fluoride in the intake water and due to the limestone used as part of the foundry process. Although GM complied with the site specific regulations adopted by the Board, in 1989 the IEPA imposed tighter mass discharge limits in GM's NPDES permit which required significantly increased recycling of process waste water. GM recently spent $1.2 million to upgrade its wastewater treatment system to comply with these stricter limits, installing a larger holding basin and upgrading its wastewater treatment process controls. As a result of these tighter limits, however, and as a result of increased levels of fluoride in the limestone, fluoride levels in the discharge began to increase in 1991.

6. In response to these increased levels, GM studied various potential sources of increased fluoride. These studies indicated that the fluoride levels in the limestone have increased above prior levels. These studies also indicated that fluoride levels in limestone fluctuate, even in limestone from the same quarry.

Compliance alternatives

7. The alternatives for achieving compliance are essentially as they were in 1981 when the site specific rule was adopted. They include: (a) additional treatment for fluoride which has significant expense and environmental consequences; (b)

discharging directly to the Vermilion River and obtaining a mixing zone; (c) locating additional sources of limestone, which involve significant transportation and other costs; and (d) site-specific relief as requested here.

8. Several treatment alternatives were outlined in the prior site specific proceeding and all of these had significant capital and operating costs. The alternatives were outlined in a Dr. James E. Etzel's testimony in the prior proceeding and the costs here are expressed in 1976 dollars. A copy of the testimony is attached and incorporated as Exhibit A. The treatment alternatives included: precipitation with high magnesium lime, with a capital cost of $6,700,000 and annual O&M costs of $1,850,000; absorption on bone char, with capital costs of $2,300,000 and annual O&M costs of $580,000; and ion exchange with activated alumina, with capital costs of $2,500,000 and annual O&M costs of $530,000. All of these alternatives would result in a high fluoride sludge which would have to be disposed in a special waste disposal facility, thus increasing the solid waste load on and diminishing useful space in that landfill.

9. The cost of constructing a conduit to transmit the discharge directly to the Vermillion River has recently been reevaluated and is estimated to exceed $460,000. This estimate does not include the cost of seeking to obtain a mixing zone from the IEPA in order to allow discharges of fluoride in compliance with the Board's effluent levels but in excess of the Board's

water quality standards. A copy of the cost estimate for this construction is attached and incorporated as Exhibit B.

10. GM has also investigated sources of low fluoride limestone. GM currently uses a limestone source 6 miles away and costs per year of purchasing and transporting limestone are $120,960. The only proven source of low fluoride limestone is in Alpena, Michigan which is 514 miles from the facility. The Alpena source produces FDA quality limestone for various products including antacids and it is the only limestone source known to GM where fluoride levels are routinely monitored. The additional cost of obtaining limestone from this source would be $541,440 per year. A potential source of low fluoride limestone is in Bloomington, Indiana, 125 miles from the plant. The excess costs for that source would be $262,656 per year. Since limestone levels from this source are not routinely monitored, it is not certain that the fluoride levels would be as consistently low as the Alpena source and therefore it is not clear that using limestone from this source will result in consistent compliance with the existing standard. A copy of the cost estimates for the costs from these sources is attached as Exhibit C.

11. It is clear that any action by GM to either treat the fluoride in its wastewater, discharge directly to the Vermillion River or obtain limestone lower in fluoride from another source would have substantial costs. Given the lack of environmental impact as described below, and the high cost of the options described above, compliance with GM's current fluoride

standard by use of any of these alternatives is economically unreasonable.

Environmental Impact

12. The facility discharges to an unnamed ditch which flows into the Vermilion River. This ditch was thoroughly studied in the R78-8 proceeding and the technical consensus at that time was that fluoride was not a limiting factor on the maintenance of a diverse aquatic community. At that time the ditch was subject to intermittent flows, had poor habitat substrate and a slope equal to a mountain stream, all of which explained the observed limited benthic community.

13. In the prior proceeding, GM witnesses testified to the impact of fluoride on aquatic life. They stated (and the Board held) that warm water midwestern aquatic communities were far less sensitive to fluoride than cold water fish such as trout and that this was explained in part by the different hardness levels and availability of alternative sources of calcium. Both GM and the Agency stated that the increased fluoride standard would have no adverse impact on any uses of the ditch or the Vermilion River and would not affect the aquatic community in either water.

14. These same conditions are prevalent now. A recent study of the stream performed by Greg Bright of Commonwealth Biomonitoring demonstrates that fluoride is not a limiting factor on the aquatic life and the habitat structure is the principal limit to achieving a more balanced aquatic community. In

addition, a recent review of the literature performed by Bright showed that increasing the fluoride limits to 10 mg/l would have no adverse impact on the fish or macroinvertebrates in the ditch or the Vermilion River. Copies of the biological assessment and literature survey are attached and incorporated as Exhibit D. The ditch remains limited by factors other than water quality and the fluoride levels to be expected in the Vermilion River will have no adverse impact on the aquatic community there.

15. GM believes that the 10 mg/l standard is consistent with GM's current discharges and necessary to prevent violations in the future. GM performed a statistical analysis of its fluoride discharges over the last three years which demonstrate that the fluoride discharges under normal operating conditions can reach a maximum of 9.75 mg/l. A copy of this report is attached hereto and incorporated as Exhibit E. GM will continue its present treatment practices which should result in average fluoride levels lower than 10 mg/l but GM is simply not able to control the levels of fluoride in its limestone which is the one variable that contributes most significantly to its increased fluoride levels. Therefore, the 10 mg/l standard is necessary as demonstrated by this statistical analysis.

16. Although GM's requested relief would increase the water quality standard for the unnamed tributary and a 0.9 mile stretch of the Vermillion River, it would also return the water quality standard for the remainder of the Vermillion river to the General Use Water Quality Standard of 1.4 mg/l. Review of recent

data suggested the lack of current need for the extended relief and that the volume of flow and structures in the Vermillion River would provide sufficient mixing so that relief beyond the 0.9 mile stretch is no longer necessary.

Justification

17. As is shown by this petition, it is not economically reasonable for GM to comply with its existing standard. The alternatives available all involve substantial expense and uncertainty, and several of them involve additional environmental consequences. Further, the relief will have no adverse impact on the receiving waters and because of its limited applicability, will not affect any other discharger. Finally, the relief will reestablish the General Use Water Quality Standard for fluoride in all but a 0.9 mile stretch of the Vermillion River.

Compliance with Federal Law

18. The Board can grant this site specific standard consistent with federal law. The USEPA has adopted no water quality criteria for fluoride and has no applicable literature concerning fluoride. The revised water quality standard would not affect any present or future uses of the receiving waters and can be adopted consistent with the requirements of the Clean Water Act and appropriate regulations.

ECONOMIC IMPACT STUDY

19. Pursuant to P.A. 87-860, Economic Impact Studies are no longer required for proposed Board regulations. Should

this requirement be modified during the pendency of this rulemaking, GM requests that the Board determine that an Economic Impact Study is not necessary. The rule change affects only GM's facility and will have no environmental impact. The Board will be in a position to determine the economic reasonableness and technical feasibility of the proposed rule based on the technical information and cost figures submitted by GM in this proceeding.

SYNOPSIS OF TESTIMONY

20. GM will call several individuals to testify in support of this Petition. They include:

A. Mr. James Schifo, Senior Process Engineer at the Danville Plant, will describe the facility and the wastewater treatment system; will outline the efforts to comply with the fluoride standard and the treatment alternatives including costs and testify that the modification of the existing standard is both economically reasonable and technically feasible.

B. Mr. Greg Bright will testify to the conditions of the receiving stream and Vermilion River and testify to the lack of environmental impact associated with the facility's discharge.

C. Ms. Dawn Cleary, Environmental Engineer at the Plant, will testify as to the beneficial economic impact of the Plant on Vermilion County and its role as a corporate citizen there.

SIGNATURES

21. Attached is a Motion to seek a waiver of the requirement to have a Petition containing 200 signatures in support of this request.

WHEREFORE for the reasons stated herein, GENERAL MOTORS CORPORATION, POWERTRAIN DIVISION respectfully requests the Board to amend the water quality standard for fluoride applicable to this facility contained in 35 Ill. Admin. Code 303.322 from 5 mg/l to 10 mg/l.

Respectfully Submitted,

GENERAL MOTORS CORPORATION,
POWERTRAIN DIVISION

By: 
One of its Attorneys

ROSS & HARDIES
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150 North Michigan
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R78-7

TESTIMONY
ON
FLUORIDE LEVELS, ACCOUNTABILITY AND TREATMENT
ON BEHALF OF THE
DANVILLE, ILLINOIS PLANT OF THE CENTRAL FOUNDRY
DIVISION OF GENERAL MOTORS CORPORATION

BY

JAMES E. ETZEL, Ph.D.

Good morning. My name is James Etzel, and I am the head of the Environmental Engineering area of the School of Civil Engineering at Purdue University and hold the position of a chaired professor. In my position I am responsible for coordination of six trained professionals all of which hold professorial rank and possess the Ph.D. degree. My academic duties consist of teaching at the undergraduate and graduate levels and directing of research. During the past 20 years I have personally supervised the graduate training of 44 masters' degree candidates and 19 Ph.D. candidates. My education consists of a Bachelor of Science degree in Sanitary Engineering from the Pennsylvania State University in 1951, and a Master of Science degree in 1955, and a Doctor of Philosophy degree in 1957, both from Purdue University. One of my other university duties is to serve as the Chairman of the Purdue Industrial Waste Conference. This conference and its published proceedings are known worldwide to the extent that many practicing environmental engineers use the proceedings as their prime reference

EXHIBIT

A

source. Along with my university activities I also serve as a consultant on industrial wastewater problems to several industrial concerns both in the United States and throughout the world. I am also a registered professional engineer in the State of Indiana and author of numerous technical articles. A copy of my vita is attached as Appendix 1.

INTRODUCTION

It is a well known fact that fluoride levels in drinking water, in the range of 0.8-1.2 mg/l, are beneficial in preventing tooth decay. For this reason, many towns in Illinois, as well as around the nation, have chosen to add fluoride to their water supply. These additions are normally made by use of the chemical compounds of sodium fluoride or hydrofluosilic acid, and, to a lesser extent, sodium silicofluoride. Choice of these particular compounds is very heavily based on their solubility, since most other compounds that contain fluoride are very insoluble. It is important to emphasize that no matter what the original compound was that contained the fluoride anion, its integrity is lost once it is dissolved in water. As an example, if one were to add 10 mg/l sodium fluoride (NaF) to tap water, all that could be said after dissolving is that the water contains an additional 5.5 mg/l of sodium cation and 4.5 mg/l of additional fluoride anion, over and above the original concentration of each ion. Previous Board deliberations that I have read concerning the fluorspar mining industry and its fluoride problem seem to be confusing on the point of calcium fluoride, since they indicate that calcium fluoride is the important material in the water. Although the presence of calcium or magnesium ions in a water containing fluoride may be significant from the standpoint of its environmental effect on fish or aquatic life, this does not mean that

calcium fluoride or magnesium fluoride as compounds are present in the water. Therefore, in collecting water quality data to be used by aquatic experts to determine any environmental effect on aquatic life, analysis cannot be performed to determine the presence of calcium fluoride or magnesium fluoride dissolved in water. Instead, samples must be analyzed for the individual ions of calcium, or magnesium, or fluoride.

With the growing trend of municipalities in the past ten years to add fluoride to drinking water, there has been a corresponding increase in fluoride levels discharged from wastewater treatment plants. Existing wastewater treatment plants, such as Blue Plains or Lake Tahoe, as well as future plans for similar advanced or tertiary wastewater treatment plants that are being designed to remove pollutants according to the most stringent pollution control regulations, are not capable of removing fluoride. It can thus be concluded that streams which have a high use rate for water supplies, and which receive high amounts of treated wastewater from any water source, should be experiencing higher levels of fluoride ion. This higher fluoride ion level is most pronounced at low stream flows, because of the high proportion of previously used water flowing in the stream at that time. This is the case with the Vermilion River at Danville, Illinois, since flow data that I have seen shows a very high proportion of the low river flows being composed of upstream sewage plant discharges.

Although data on the fluoride content of the Vermilion River,

either upstream of the foundry intake point or at the intake point itself, are not available in mass, I have assembled a combination of data and analyses from GM's various contractors, the Illinois Water Survey and the IEPA, which are compiled in Table 1 of this document. Using these data, it was possible to produce a probability plot as to the percentage of time that a given fluoride level could be expected to be equaled or exceeded. This plot is shown in Figure 1 in this document, and the data relative to decennial percentages and their corresponding fluoride concentrations in the Vermilion River are presented in Table 2. These data show that on the average a fluoride level of 0.8 mg/l or greater can be expected 50% of the time. This fluoride level is in the exact range which would be expected because of the high usage rate of the Vermilion River as previously pointed out.

IN-PLANT CONSIDERATIONS OF FLUORIDE SOURCES

As has already been described, the Danville plant of the Central Foundry Division of the General Motors Corporation has devoted a lot of time, effort and money to the subject of fluoride sources within its process materials. Through changes in raw materials such as fluorspar, it was thought that enough of the fluoride sources had been eliminated so that the fluoride levels in the plant wastewater discharge would no longer be a problem. However, extensive testing was conducted to develop a treatment system that would treat a major portion of the plant wastewaters for heavy metals and suspended solids down to Board limitations, and this testing showed that the changes in raw materials did not eliminate the problem of fluoride levels at 002. During this testing, a few analyses of the main discharge from the plant (002) were run for total and soluble fluorides, more or less as a check to confirm that the concentrations were not a factor. To everyone's surprise, a few spot-check fluoride analyses by a GM contractor showed values in the range of 1-3 mg/l. This data suggested that a much closer look at the sources of fluoride would be necessary to ascertain the origin of the fluorides. A study of the problem led to a report on "Fluoride Sources Within the Production Processes and Manganese Levels in Discharge 003" in October, 1977, which is attached as Exhibit G to GM's regulatory petition in this proceeding. This report showed the major

sources of fluoride to be the limestone used in the cupola charging and the Vermilion River, as well as the Interstate Water Company's fluoridated water.

Using the data from Table 2 on the intake water from the Vermilion River and Table 4 on the plant discharge at outfall 002, one can see that, 50% of the time, the 0.8 mg/l of soluble fluoride in the plant intake water is 40% of the 2 mg/l soluble fluoride at point 002. Looking into all the other process materials, that are used in any quantity large enough to be significant, showed the limestone flux, the coke, and the anthracite coal, all used in the cupola charges, to be the only likely sources of the fluoride. An analysis of the coal showed it to contain 0.005% by weight of fluoride, and, since the coke was more pure than the coal, it was dismissed as a possible source. Materials balance calculations on the cupolas showed the coal at 0.005% fluoride to be an insignificant source. Tests on the limestone showed that it contained 0.04% by weight of fluoride, and, since it is used in such mass quantities (100+ tons/day), the materials balance calculations across the cupolas showed the limestone to be a major factor in the source of fluorides. Using a 002 discharge flow of 10.12 mgd, the basis for which I will explain later, and a hypothetical soluble fluoride concentration of 1.22 mg/l [2.00 mg/l, the 002 effluent 50% of the time, minus 0.78 mg/l, the intake water level 50% of the time], approximately 103 pounds of fluoride would be added to the water on such a hypothetical day. In comparison, the plant's limestone usage, during

the period in which I conducted my fluoride mass balancing tests at the plant, averaged 141 tons/day, which, with a 0.04% fluoride content in that limestone, liberated approximately 112 pounds of fluoride. The close agreement of these numbers is very strong data to support the statement that the water source and the limestone were the two major fluoride sources.

ALTERNATIVES TO ELIMINATE OR REMOVE FLUORIDE

Since the only controllable source of fluoride was the limestone flux, it appeared that an alternate source of limestone, which was lower in fluoride content, should be considered. Calculations to determine how low the fluoride content of the alternate limestone would have to be were the first order of investigation. Referring to Table 2, it becomes clear that with a probable river intake level of soluble fluoride of 1.32 mg/l or greater 10% of the time, an effluent limit of 1.40 mg/l 100% of the time is impossible. An actual plant-water balance study, as presented in Bob Jones' testimony, showed that the 11.016 mgd of Vermilion River water, together with 0.895 mgd from the Interstate Water Company, made up the total plant water usage on a typical operating day in November, 1976. Of this water intake, 0.120 mgd was discharged as sanitary sewage to the Tilton Sanitary District; 0.091 mgd was discharged via outfall 001; and 0.0035 mgd was discharged via outfall 003. This total water discharge of 0.2145 mgd, or about .22 mgd, had no effect on discharge 002, nor was it subjected to much evaporation. The remaining flow of approximately 11.68 mgd [11.9 mgd minus 0.22 mgd] was used in ways where evaporation losses of 1.56 mgd occurred. Thus, the discharge at 002 had a volume of approximately 10.12 mgd, but contained all of the dissolved and suspended constituents in the

11.68 mgd of original water. If we thus consider, from Table 2. the 10% probability value of 1.32 mg/l of fluoride in the Vermilion River intake water, and apply a concentration factor for evaporation based upon the above water balance data, we would get a soluble fluoride value in discharge 002 of approximately 1.5 mg/l. These facts show that, without any fluoride additions by the plant, meeting a discharge limitation of 1.4 mg/l is impossible.

All of this shows you what the situation is without any fluoride additions by the plant. But let me now tell you what happens when the plant's use of limestone in the process adds fluoride to the plant's Vermilion River intake water. For example, at a usage rate of 141 tpd of limestone, which was the plant's average usage during the period in which I conducted the fluoride mass-balancing tests, and at a fluoride content in that limestone of 0.04%, there would be a total fluoride release of 119 pounds/day added to the water. At this level, assuming a discharge flow at 002 of 10.12 mgd, an added fluoride concentration of 1.41 mg/l would result from the limestone. Using the data in Figure 1, on the soluble fluoride content of the Vermilion River intake water, we can thus calculate the possible fluoride levels at 002. Using the 99% probability value of 1.78 mg/l soluble fluoride, and correcting for evaporation loss, a value of 2.05 mg/l results. Adding the 1.41 mg/l and 2.05 mg/l values yields 3.46 mg/l of soluble fluoride in discharge 002. In the same way, the 99.9% soluble fluoride at 002 would be 2.42 plus

1.41, or 3.83 mg/l or higher. The 99.99% value would be 4.18 mg/l or higher. However, as Bob Jones has pointed out, the plant's limestone usage rate has reached 175 tpd, a level well in excess of the above rate of 141 tpd; and this higher usage rate would yield a total fluoride release of 140 pounds/day, or 1.66 mg/l. Again using the 99.99% probability value of river intake water, a soluble fluoride value of 4.59 mg/l or higher could result at discharge 002. Accordingly, it is easy to understand why a 5 mg/l limitation for fluoride is necessary at outfall 002.

In spite of this situation an investigation into other possible limestone sources lower in fluoride was conducted. After many contacts with state geologists in Illinois and Indiana, along with contacts at Purdue, the University of Illinois, and Indiana University departments of geology, no data on fluoride contents of limestone could be found. This lack of data was understandable, because most quarries do not typically analyze for constituents which would be present in trace amounts, such as fluoride. Some opinions that 0.04% by weight was not high, or seemed low, were all that could be obtained. Analyses of three other limestone sources were then pursued. One source near Kentland, Indiana, showed a limestone with a fluoride content of 0.074% by weight. One near Fairbury, Illinois, showed a value of 0.057% by weight. And one from Thornton, Illinois, showed a value of 0.02% by weight, suggesting that use of this limestone would cut fluoride contributions by

the plant, due to limestone, to half of their existing levels. However, such a reduction would not allow the plant to meet the existing 1.4 mg/l limit, because of the probability values as to the Vermilion River intake water, and the evaporation rate, that I just mentioned. In fact, applying the above limestone usage rates of 141 tpd or 175 tpd, using the Thornton limestone would reduce the plant's fluoride contribution to 0.70 or 0.83 mg/l, respectively. Then, adding these values to the above probability values of the Vermilion River intake water, yields a fluoride concentration range at 002 of 2.75 to 3.60 mg/l or higher. This information, together with the cost impact of such a limestone substitution as presented by Bob Jones, clearly leads to the conclusion that limestone substitution would not allow the plant to comply with the existing 1.4 limit, and in any case is not an economically reasonable alternative for reducing fluoride levels at 002.

Consideration was also given to the discharge of the wastewater from outfall 002 directly into the Vermilion River via a pipeline. The data on river flow and on fluoride levels are very much parallel, and so, as river flow decreases, the fluoride and soluble fluoride concentrations increase. Taking into account that the seven day ten year low flow at Danville is 13.2 mgd, it can be seen that no dilution water for discharge 002 would be available (an upstream withdrawal of 10-12 mgd must be taken into account), and thus no benefits could be derived from direct river discharge by means of a pipeline.

It was thus concluded at this point in the investigations that neither materials substitution nor pipeline discharge were feasible means of meeting the applicable fluoride limitation.

FLUORIDE REMOVAL BY PHYSICAL OR CHEMICAL TREATMENT

In evaluating the treatment alternatives for removal of the heavy metal ions in the plant wastewater flow, such processes as reverse osmosis, high pH chemical precipitation with lime, and ion exchange were considered. In the case of either reverse osmosis or ion exchange, one is still left with what to do with the reverse osmosis reject water, or the ion exchange regeneration wastewater, both of which would contain a high concentration of total dissolved solids. Considerations of using lime precipitation showed that about 13.5 tons/day of lime would be consumed since all of the 350 mg/l of hardness as $CaCO_3$ would have to be removed along with the heavy metal ions. Not only would this have cost the environment a lot of energy to produce the lime, but also the 164,000 gal/day of 5% total solids sludge that would result would have been a significant problem to dispose of in an environmentally acceptable manner. Consideration of ion exchange showed that the quantity of lime would not have been reduced since treatment of the regeneration wastewater was necessary and a quantity of approximately 42 tons/day of salt (NaCl) would have entered the environment in addition. It was on the basis of this information that Central Foundry Divsion of the General Motors Corproation decided to try a totally new and undemonstrated technology. The technology employed the use of alum coagulation for removal of colloidal materials and made use of the fact that at pH values of about 9 the hydrous oxide flocs of aluminum have ion exchange

properties. Laboratory scale tests of the technology showed it to be very promising and formed the basis for full scale plant construction. Data on the full scale system has already been presented by Bob Jones, but I emphasize that the low dosages of alum and sodium hydroxide, along with the resulting sludge, are minimal compared to any of the other technologies considered.

An in depth look at fluoride removal methods showed that only the following technologies were applicable: adsorption on bone char; ion exchange with activated alumina; and precipitation with high magnesium lime. However, none of these technologies can reduce soluble fluoride levels below 1.0-1.5 mg/l, and I would not guarantee that any of them could achieve consistent compliance with the limit of 1.4 mg/l. Moreover, the costs of each technology are extremely high, both as to capital investment and annual operating costs; and each technology produces large volumes of sludge, the disposal of which is also very costly as well as environmentally unacceptable. In addition, one must account for the pollution caused in the manufacture of the very chemicals needed to apply this technology.

For example, in using bone char technology, it would first be necessary to have a coagulation and settling system as has recently been installed at the Danville plant. The coagulated and settled water could then be passed through the bone char for fluoride removal, and then the defluoridated water would require filtration. The last filtration would be required because

of the high attrition losses of the bone char and the effect these would have on suspended solids and fluoride in the final effluent. Further, there would have to be a regeneration of the bone char using sodium hydroxide. The resulting regeneration wastewaters would contain the same mass of fluoride as originally present, but in a more concentrated form. It would therefore be necessary to use lime treatment with high-magnesium lime, to ultimately remove the fluoride from the regeneration wastewaters. In addition, this treatment of the regeneration wastewater would produce more than 14,000 gpd of sludge, which would also have to be disposed of in some environmentally acceptable manner.

The expense of bone char exchange, above and beyond the capital and operation expense of the new wastewater treatment plant that went on-line on September 1, would require a capital cost of $800,000 and an annual chemical cost of $250,000. The final capital cost for regeneration wastewater treatment and sludge dewatering would run approximately $1.5 million with annual chemical costs of $180,000, and sludge disposal costs of another $150,000 per year. As a grand total, and above and beyond the capital and operating expense of the new wastewater treatment plant, capital costs for bone char treatment would be $2.3 million and annual chemical and sludge disposal costs would be about $0.58 million. These estimates, and the others I will give you shortly, are based on my knowledge of the required hardware and equipment, together with information I have obtained

through calls and contacts to suppliers and other industry representatives. My figures include all project costs, but do not include operating labor. All of these costs, as you can see, would be for something that is borderline in meeting the required water quality limits at outfall 002.

Activated alumina exchange would have the same pretreatment requirements as those for bone char. While the bone char technology is an adsorption process, and the activated alumina is an ion exchange process, both are similar in their requirement of regeneration. The wastewaters resulting from regeneration of activated alumina thus contain the same mass of fluoride as originally present, but in a more concentrated form--and now have to be treated with chemicals for precipitation. The only difference between the two technologies is that a filtration is needed prior to exchange, rather than after, because of the fine nature of the activated alumina. The expense for activated alumina, above and beyond the capital and operating expense of the new wastewater treatment plant, would require a capital cost of about $1.0 million, and an annual chemical cost of about $0.2 million. The regenerant treatment and sludge dewatering costs would be essentially the same as those for the bone char: capital $1.5 million and annual chemical and sludge disposal costs of $0.33 million. Total capital costs, above and beyond the capital and operating expense of the new wastewater treatment plant, would be $2.5 million with annual chemical and sludge disposal costs of $0.53 million, excluding operating labor.

When we were considering the various alternatives that could be used to remove the heavy metals in the 002 wastewater discharge, high pH precipitation was considered and abandoned for a variety of reasons. It seemed possible, however, that if high pH precipitation could be used to remove heavy metals and soluble fluoride, it might be worthy of a second evaluation. Technology showed that a magnesium lime, instead of a regular high calcium lime, would be necessary; and that the process would be considerably different than the one recently installed. The start of treatment would be with chemical addition, rapid mix, flocculation, and then settling in conventional settling tanks, instead of a lagoon as in the new plant. Tanks for settling would be required since the sludge volume of 164,000 gpd was so large that it required constant removal rather than annual cleaning as does the existing lagoon. Sludge recycle back to the rapid mix tank would be needed to minimize scaling and sludge volume. The settled effluent would have to be stabilized by recarbonation or acid addition prior to filtration so as not to scale the filters or have any after-precipitation in the stream due to super saturation with calcium carbonate or magnesium hydroxide.

Capital costs for the high magnesium lime precipitation were found to be $5.5 million. Annual chemical costs for high magnesium lime and sulfuric acid to control pH after the precipitation were $0.35 million. Dewatering of the 164,000 gallons per day of sludge which would be produced from the precipitation, and disposal of the dewatered cake, would require another $1.2 million of capital, and $1.5 million of operating costs exclusive of labor. A grand total of $6.7 million of capital, and $1.85 million of operating costs exclusive of labor, would be needed.

During the evaluation of the fluoride removal processes the technology of using magnesium as a co-precipitant for fluoride in the high magnesium lime process seemed as though it might have a chance of working if used in another location. The rationale was that if dolomitic limestone (high magnesium limestone) could be added to the high calcium limestone in the cupola charge in a ratio where the magnesium content was high enough to cause co-precipitation of the fluoride, the fluoride might not be solubilized in the wet cap and scrubbing waters used in the cupola. A study of this idea involving full scale tests on one of the cupolas was made and a report "Dolomite Limestone Study on Control of Cupola Fluoride Losses" was prepared in July, 1978. Using the equations for fluoride removal by co-precipitation with magnesium, a charge of 50 pounds of dolomitic limestone per cupola charge should have been more than enough to drive the soluble fluoride in the cupola wastewaters to zero. The thought behind the use of dolomitic limestone was that, in the molten fluid mass of the cupola, the reactions between fluoride and magnesium would take place much as they do in a water environment. Thus the fluoride would be chemically bound to the magnesium and leave the cupola by way of the slag. Another possible action was the evolved fluoride and magnesium, when removed from the cupola off-gases by the venturi scrubber, would react to co-precipitate in the water. Tests showed little or no change in the soluble fluoride content of the cupola wastewaters. A further attempt using 100 pounds of dolomitic limestone per cupola charge (a 200% excess) was made, but the results still gave no indications of fluoride removal, and so further testing of the approach was abandoned. Other considerations

in using the dolomitic limestone took into account the fact that oliflux, a magnesium oxide material which had been previously used in the cupola charge, could be substituted for the dolomitic limestone had it been successful, without the fear of a significant increase in operating costs for obtaining and using dolomitic limestone, as previously pointed out by Bob Jones.

STATUS OF PRESENT TREATMENT SYSTEM REMOVALS

The new technology wastewater treatment system has, as already shown, demonstrated a very high degree of heavy metal ion removal ability. Data in Table 5 on the concentrations of total and soluble fluoride in the Vermilion River, treatment system influent (Parshall flume), and outfall 002 clearly show that the system has no effect on soluble fluoride anions. This fact is exactly as expected since only cation exchange capacity is possible with the hydrous oxide aluminum floc. The slight variations between soluble and total fluoride levels in Table 5 are normal experimental error and any removals of soluble fluoride across the sytem are a matter of chance since influent and effluent samples were not staggered to allow for system detention time. While these data are not exhaustive, they are, in my opinion, sufficient to show if any unexpected removals were being achieved. Since no removals were shown, it can be concluded that soluble fluorides, and more than likely no anions, are removed or exchanged by the wastewater treatment system.

SUMMARY

A new technology wastewater treatment system has, during its early operational history, been shown to be capable of achieving high degrees of heavy metal cation removal, but no ability to remove or exchange anions. Other investigations involving dolomitic limestone additions to the high calcium limestone flux to the cupolas proved ineffective in reducing or eliminating the soluble fluorides in the cupola wastewaters.

Probability studies on the levels of soluble fluorides in the Vermilion River showed that levels higher than 1.4 mg/l could occur by chance alone at least 7% of the time. Allowing for the evaporation losses in the process, a level of soluble fluoride of only 1.2-1.3 mg/l could be in the Vermilion River water if a level of 1.4 mg/l were not to be exceeded in discharge 002. This means that the per cent of time for exceeding the 1.4 mg/l level in discharge 002 would be ~~11~~ 16%. All of the foregoing data assume zero fluoride ion is added in the plant processes. Correlation of the probability data on fluoride levels with Vermilion River flows, coupled with water withdrawals from the river, show that pipeline discharge directly to the river would be of no advantage because of the absence of dilution water in the river.

In depth evaluations of fluoride removal processes showed that they were only good enough to reduce the discharge level at point 002 to about 1.0-1.5 mg/l of soluble fluoride. This fact, when coupled with the possibility of creating masses of poorly dewaterable sludge and possible adding of up to 42 tons per day of salt to the river flow, seemed to be more environmentally detri-

mental than the minimal levels of soluble fluoride that now exist in discharge 002. It is on the basis of this information and in the interest of environmental preservation that I feel a legal remedy rather than a treatment system is the most reasonable solution to the fluoride situation.

CONCLUSION

1. Meeting a fluoride level of 1.4 mg/l is impossible when we consider the fluoride levels that occur in the Vermilion River intake water.

2. Use of an alternate limestone for the current one, in order to achieve compliance with the 1.4 limit is impossible, since no limestone to my knowledge is free of fluoride.

3. Changes in cupola charge materials to eliminate fluoride losses have been unsuccessful in meeting the 1.4 limit.

4. Removal of fluoride by known technology cannot guarantee continuous compliance with a limit of 1.4 mg/l.

5. Damage to the environment from production of chemicals used in fluoride removal and by sludges resulting from the removal are more than those from the original fluoride.

JAMES E. ETZEL

Education

1957 Purdue University, Lafayette, Indiana,
Ph.D., Civil Engineering

1955 Purdue University, Lafayette, Indiana,
M.S., Civil Engineering

1951 Pennsylvania State University,
B.S., Sanitary Engineering

Present Professional Activities

Dr. Etzel is currently a Chaired Professor and Head of Environmental Engineering area, School of Civil Engineering, Purdue University. In this capacity he directs a broad program of environmental control research in solid wastes, air, and water pollution control technology. Dr. Etzel is Chairman of the Purdue Industrial Waste Conference, an internationally recognized symposium that annually attracts hundreds of industrial and regulatory participants concerned with the development of the best available control technologies for a wide variety of industries. At the University, and as a consultant, he is actively engaged in directing laboratory, pilot plant, and commercial operations in regard to air and water pollution control and regulatory compliance. In addition to Flow Resources, Dr. Etzel provides consulting services to E.I. duPont, General Motors Corp., Monsanto Chemical Co., ITT, Water Refining Co.,Union Carbide, and Inland Container Corp.

Past Professional Experience

Over the past twenty years, Dr. Etzel has been associated with Purdue University's Environmental Engineering Program, and has served as Director of the University's Environmental Institute. Prior to his association with Purdue, Dr. Etzel was Director of Research and Development, P.F. Weston, Inc., Consulting Engineers; Industrial Wastes Engineer, E.I. duPont; and Assistant Operations Officer, U.S. Army Corps of Engineers.

Recognition, Awards, Honors

Dr. Etzel is a Registered Professional Engineer (Indiana, PE11043), and has been the recipient of numerous awards and honors, including the Harold Munson Award for being the outstanding teacher for 1978 in the Purdue University School of Civil Engineering. Aside from his capacity as Chairman of the Purdue Industrial Waste Conference, he is a Past President of the Indiana Water Pollution Control Association. He is listed in Who's Who in the Midwest and is a member of numerous professional organizations.

Publications

Best Paper Awards and author of numerous articles on industrial waste treatment.

SOLUBLE FLUORIDE CONCENTRATIONS IN THE VERMILION RIVER AT DANVILLE, ILLINOIS, UPSTREAM OF OR AT THE WATER INTAKE POINT OF CENTRAL FOUNDRY DIVISION
1974-1978

Soluble Fluoride mg/l	Mid Point of Percent
0.2	1.43
0.2	4.29
0.3	7.15
0.3	10.01
0.3	12.87
0.3	15.73
0.3	18.59
0.3	21.45
0.3	24.31
0.3	27.17
0.4	30.03
0.4	32.89
0.6	35.75
0.6	38.61
0.7	41.47
0.8	44.33
0.8	47.19
0.9	50.05
0.9	52.91
0.9	55.77
0.9	58.63
0.9	61.49
0.9	64.35
0.9	67.21
0.9	70.07
1.0	72.93
1.1	75.79
1.1	78.65
1.1	81.51
1.2	84.37
1.2	87.23
1.3	90.09
1.4	92.95

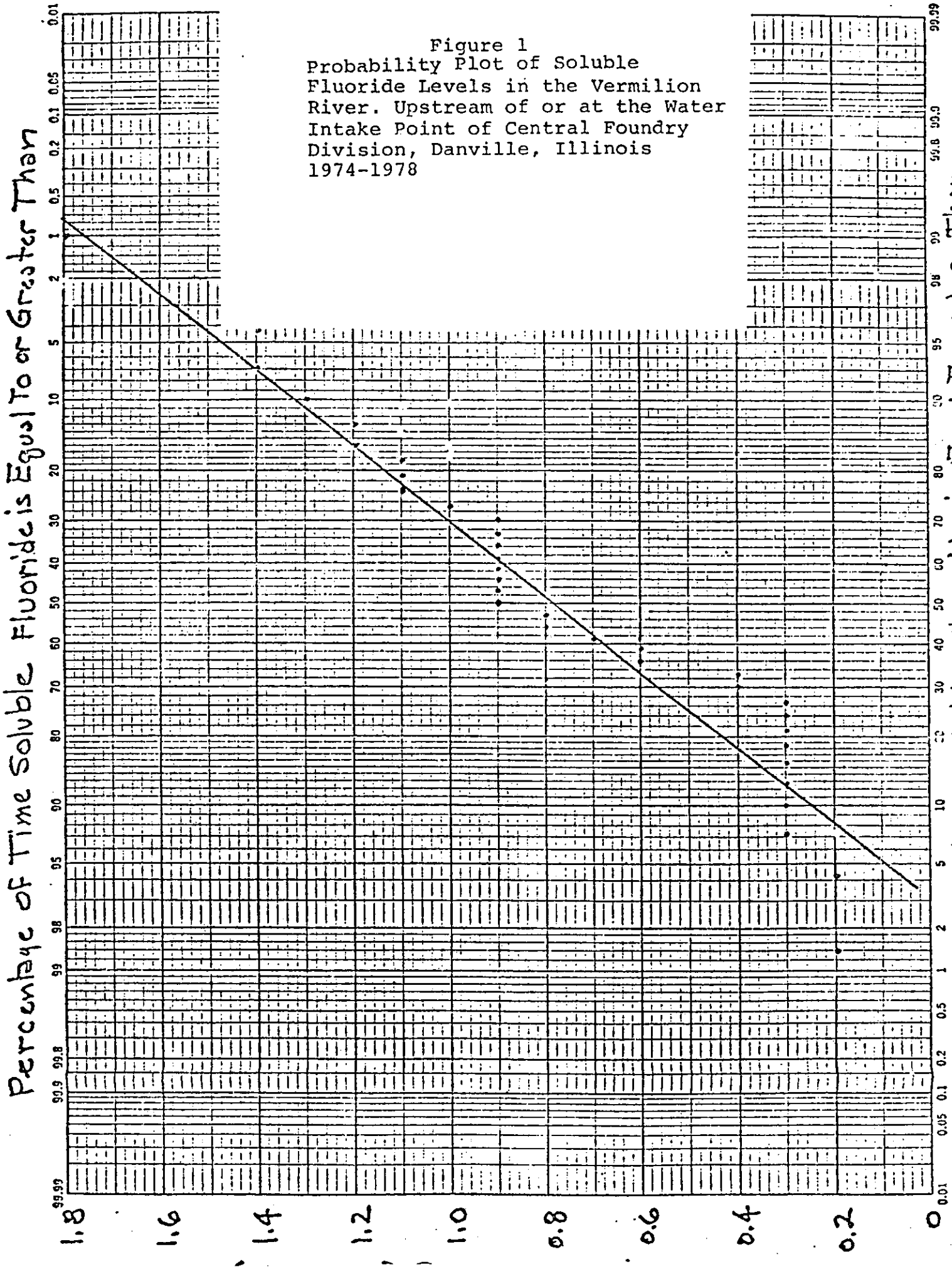


Figure 1
Probability Plot of Soluble Fluoride Levels in the Vermilion River. Upstream of or at the Water Intake Point of Central Foundry Division, Danville, Illinois 1974-1978

Time-Concentration Relationship for Soluble Fluoride in the Vermillion River at Danville 1974-1978

Percentage of Time Soluble Fluoride Concentration Will be Greater Than a Given Value	Soluble Fluoride Concentration in mg/l
90	0.25
80	0.43
70	0.56
60	0.68
50	0.78
40	0.88
30	1.00
20	1.13
10	1.32

Table 2

Soluble Fluoride Concentrations in Outfall 002 of Danville Plant Central Foundry Division of GMC 1974 - 1978

Soluble Fluoride mg/l	Mid Point of percent
0.9	1.35
1.2	4.05
1.3	6.75
1.3	9.45
1.5	12.15
1.5	14.85
1.6	17.55
1.6	20.25
1.6	22.95
1.7	25.65
1.7	28.35
1.7	31.05
1.8	33.75
1.8	36.45
1.8	39.15
1.9	41.85
1.9	44.55
1.9	47.25
2.0	49.95
2.0	52.65
2.1	55.35
2.2	58.05
2.2	60.75
2.2	63.45
2.2	66.15
2.2	68.85
2.3	71.55
2.3	74.25
2.3	76.95
2.4	79.65
2.4	82.35
2.4	85.05
2.4	87.75
2.6	90.45
2.6	93.15
2.6	95.85
2.8	98.55

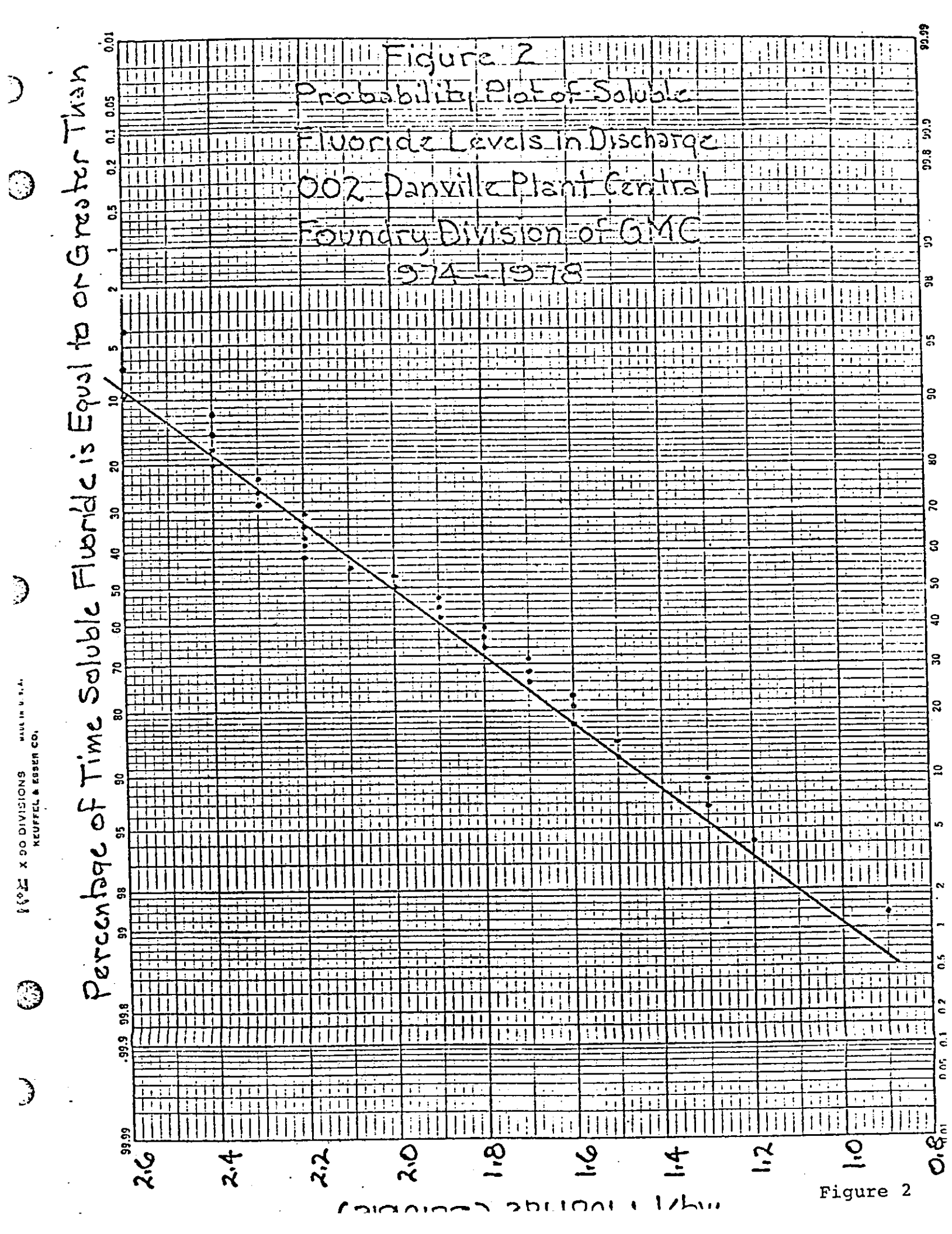


Figure 2
Probability Plot of Soluble
Fluoride Levels in Discharge
002 Danville Plant Central
Foundry Division of GMC
1974 - 1978
Percentage of Time Soluble Fluoride is Equal to or Greater Than
0.01
0.05
0.1
0.2
0.5
1
2
5
10
20
30
40
50
60
70
80
90
95
98
99
99.8
99.9
99.99
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8

Figure 2

Time-Concentration Relationship for Soluble Fluoride in Discharge 002 Danville Plant of Central Foundry Division of GMC 1974-1978

Percentage of Time Soluble Fluoride Concentration Will be Greater Than a Given Value	Soluble Fluoride Concentration in mg/l
90	1.44
80	1.64
70	1.78
60	1.90
50	2.00
40	2.12
30	2.24
20	2.37
10	2.57

GMPT 002 DISCHARGE TILE 03/12/93
COST ESTIMATE

EXTEND 002 DISCHARGE DIRECTLY TO THE VERMILION RIVER.

ITEM	COST
CLEAR BURSH	$2,000
LAYOUT SEWER	$1,800
4,250 FT. OF 48" TILE	$294,000
LAY TILE	$66,000
INSTALL MANHOLES	$40,000
BACKFILL	$10,550
HEADWALL	$3,450
EQUIPMENT MOVES	$1,350
SUBTOTAL	$419,150
+10% MISC. & UNFORSEEN	$41,915
GRAND TOTAL	$461,065

EXHIBIT

B

GM POWERTRAIN, DANVILLE FOUNDRY
ALTERNATE LIMESTONE SOURCES

04/19/93

ITEM	CURRENT SOURCE FAIRMOUNT,IL.	PROVEN LOW FLUORIDE SOURCE ALPENA, MICH.	POTENTIAL SOURCE (INSUFFICIENT TESTING) BLOOMINGTON, IN.
USAGE (TONS/YEAR)	23,040	23,040	23,040
DISTANCE (MILES)	6	514	125
PENALTY (MILES)	0	508	119
PENALTY PER TON	$5.25	$28.75	$16.65
COST PER YEAR	$120,960	$662,400	$383,616
COST PENALTY PER YEAR		$541,440	$262,656

NOTES:
FLUORIDE TESTING FROM BLOOMINGTON, IN. IS NOT SUFFICIENT TO QUALIFY IT AS A LOW FLUROIDE SOURCE. LIMITED TESTING INDICATES THAT IT WARRENTS FURTHER CONSIDERATION.

TOTAL P.02

EXHIBIT
C

Review of Potential for Environmental Impact from Fluoride in Wastewater

GM Powertrain Outfall 002
Danville, Illinois

EXECUTIVE SUMMARY

Commonwealth Biomonitoring conducted two studies associated with fluoride in the wastewater of GM Powertrain outfall 002: a literature review of fluoride toxicity and a rapid bioassessment of use impairment in the company's receiving stream. The purpose of the studies was to evaluate any environmental impacts from fluoride in the company's discharge.

The hardness of the receiving stream below outfall 002 is typically greater than 300 mg/l. A review of fluoride toxicity information from previously published scientific literature supports a conclusion that 10 mg/l of fluoride in water of this hardness should not adversely affect freshwater organisms.

The bioassessment study showed that the stream below outfall 002 is moderately impaired. Much of the impairment is probably due to high dissolved solids from coal spoils runoff in the watershed. Typical toxicity-related effects were not observed. The study supported the literature search cited previously in showing that typical fluoride levels in this stream are probably not high enough to cause toxicity problems to resident aquatic species.

Neither the receiving stream nor the Vermilion River downstream from GM Powertrain is used as a public water supply or as a significant source of stock watering. Therefore, potential for fluoride-induced mottling of tooth enamel from the discharge is low.

GM is requesting that the NPDES permit limit for fluoride be raised to 10 mg/l. Both field and laboratory studies support the conclusion that this limit would not cause environmental harm.

EXHIBIT

D

AQUATIC BIOLOGICAL ASSESSMENT
GM POWERTRAIN RECEIVING STREAM
DANVILLE, ILLINOIS
DECEMBER 1992

Prepared for:

GM Powertrain
Danville, Illinois

Prepared by:

COMMONWEALTH BIOMONITORING
7256 Company Drive
Indianapolis, Indiana 46237
(317)887-5855

AQUATIC BIOLOGICAL ASSESSMENT
GM POWERTRAIN RECEIVING STREAM
DANVILLE, ILLINOIS
DECEMBER 1992

I. Introduction

GM Powertrain manufactures automotive parts and discharges wastewater from outfall 002 to the extreme headwaters of an unnamed tributary of the Vermilion River. The company's wastewater contains fluoride derived from limestone used in the manufacturing process. The particular source of limestone used by GM Powertrain is relatively high in fluoride content, which causes the fluoride concentration in the wastewater to periodically exceed the company's NPDES permit limit of 5 mg/l.

The fluoride limit in the company's present NPDES permit was based on an Illinois Pollution Control Board rule supported by fluoride toxicity literature and biological data showing that 5 mg/l would not cause environmental harm. Based on increased fluoride in the limestone used in the foundry process, the company believed that the 5 mg/l limit did not appear to be economically achievable and they wanted to determine whether the limit was more restrictive than necessary to protect designated uses of the stream. The company then commissioned this study.

This aquatic biological assessment was conducted to determine whether fluoride periodically exceeding 5 mg/l in the GM Powertrain effluent was adversely affecting the aquatic community of the stream. If no effects from fluoride were observed, the evidence provided by a field study would help support evidence from laboratory toxicity studies (submitted separately) that fluoride in the company's wastewater was not environmentally significant.

II. Objectives

An aquatic biological assessment of the GM Powertrain receiving stream at Danville, Illinois was conducted during December 1992. The study was designed to help determine the potential environmental effects of fluoride in the GM Powertrain effluent. The scope of work was read and approved by Illinois EPA before the assessment was made.

III. Description of the Area

The unnamed GM Powertrain receiving stream is a first order tributary of the Vermilion River on the south edge of Danville, Illinois. The stream drains an area of approximately 3 square kilometers and its watershed includes both industrial, residential, and forested areas. The immediate area of the stream is heavily wooded and the channel is relatively unaltered. Stream slope is very steep, dropping approximately 30 meters in its 1 km length. This steep gradient causes the stream to fall in a series of short waterfalls and debris dams (up to 1 m high), and a bedrock substrate exists in many places. The stream is 1-3 meters wide, with pools up to 1 m deep.

The relatively natural, wooded surroundings of the receiving stream are interrupted by numerous areas of old coal mining spoils in the immediate area. The watershed was probably mined near the turn of the century, but seeps of runoff water from these spoils were observed thoughout the entire length of the stream. A sample of water from one of these seeps had the following chemical characteristics:

pH	7.7
conductivity	1060 uS
hardness	430 mg/l
alkalinity	248 mg/l
sulfate	325 mg/l
chloride	145 mg/l
sodium	220 mg/l

These chemical concentrations are characteristic of old coal spoils. Runoff from the spoils significantly boost the ionic composition of the surrounding surface waters compared to unimpacted streams.

IV. Methods

The methods used for bioassessment were adapted from the U.S. EPA Technical Support Document Rapid Bioassessment Protocols for Use in Streams and Rivers (EPA/444/4-89-001, Plafkin et al. 1989). Bioassessment Protocol III for benthic macroinvertebrates was used for the study. Protocol III requires qualitative sampling of benthos by kicknet from a riffle area, identification to the genus or species level of 100 individuals from each site, functional feeding group analysis, and sampling from an unimpacted reference stream. Data from the reference stream are used for comparison to determine whether uses for aquatic life have been impaired.

To help separate habitat effects from water quality effects, Protocol III requires that habitat in study and reference streams be as similar as possible. Riffle areas were relatively uncommon in the study stream, but water flowing over exposed bedrock was common. Therefore, all benthos samples were collected similarly from bedrock outcrops where current speed approached 30-40 cm/sec. Copies of all habitat evaluation forms are attached in an Appendix. A discussion of stream habitat effects are included in the Results section (Section V, below)..

Samples were collected in the GM receiving stream about 100 m downstream from outfall 002. In addition, another sample was collected from a second unnamed tributary to this stream, entering about 200 m downstream from outfall 002. This unnamed tributary had habitat which was very similar to the GM receiving stream and it drained much of the same larger watershed. The Town of Tilton once had a municipal wastewater discharge to this stream, but the town discontinued its discharge at least three years ago and the stream no longer has any point source wastewater discharges. However, because it drained areas potentially affected by coal mine spoils (see discussion below), the aquatic community of this stream would probably be representative of that expected in the GM receiving stream if no wastewater discharge occurred there.

Willow Creek, a first order tributary of the Vermilion River about 10 km southeast of the GM Powertrain property, was chosen as the reference stream for this study. Willow Creek is a reasonably natural stream, with an 8 square kilometer watershed, about 50% of which is forested and which has relatively little human habitation. The stream receives no point source wastewater discharges and has not been extensively channelized. The sampling site was located in Forest Glen Park, a nature preserve and recreational area owned by Vermillion County. Willow Creek lies within the same "Central Corn Belt Plains" ecoregion as the GM Powertrain receiving stream.

For quality assurance purposes, a duplicate sample was collected at the Willow Creek site. Analysis of this duplicate was used to determine the amount of variability associated with the sampling technique and the resulting biometrics.

A generalized map showing the collecting sites and GM Powertrain's outfall 002 are shown in Figure 1.

V. Results

HABITAT EVALUATION

All streams in the study had similar aquatic habitats (see Appendix for habitat assessment field sheets). The habitat scores for each stream were as follows:

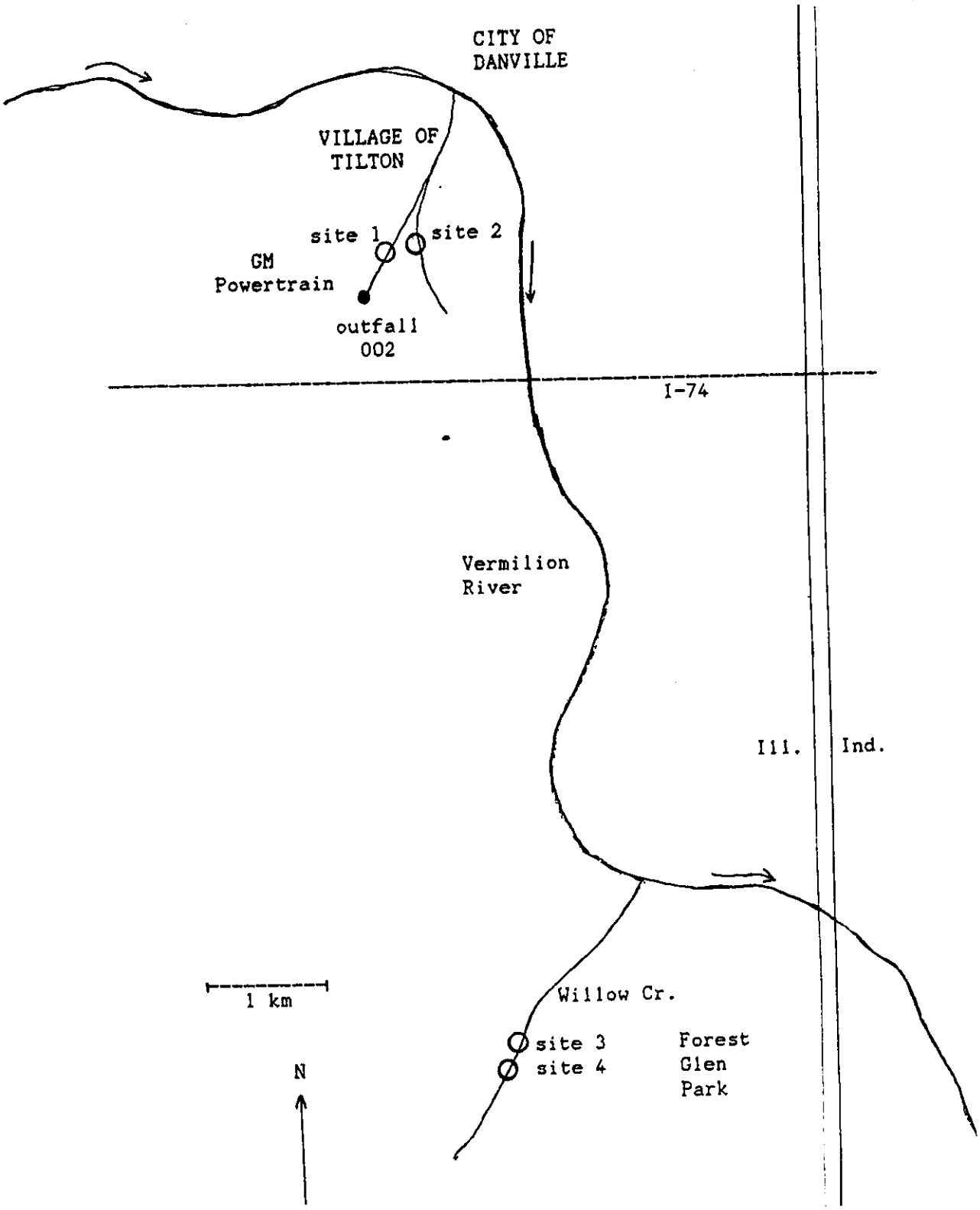
Unnamed GM Receiving Stream	84 out of 135
Tributary to Receiving Stream	81 out of 135
Willow Creek (reference stream)	93 out of 135

According to the EPA Habitat Evaluation Scheme, which ranks habitat as Excellent, Good, Fair, and Poor, each of these streams had "Good" aquatic habitat. The score for the reference stream, Willow Creek, was 11 to 15% higher than either of the study streams. It appeared to have slightly less channel alteration and bottom deposition than the two study streams. However, the difference in habitat scores was probably not large enough to account for the impaired aquatic communities discussed below.

QUALITY CONTROL DUPLICATE

The quality control duplicate samples at Willow Creek (Sites 3 and 4) indicated that the biometrics at these sites were very similar (Table 5). The bioassessment scores varied by less than 13% and both scores indicated no impairment of uses in the reference stream. Therefore, results of bioassessment scores at the study sites can probably be accepted with a high degree of confidence.

Figure 1.
Location of Sampling Sites



IMPAIRMENT

Macroinvertebrate sampling results for each site are shown in Tables 1-4. A summary of the biometrics and bioassessment scores for each site are shown in Table 5. These bioassessment scores are based on comparison to reference site 3 on Willow Creek.

The U.S. EPA Rapid Bioassessment Protocol III defines impairment of uses for aquatic life as follows:

Study Site / Reference Site Score	Impairment Condition
>0.87	None
0.54 - 0.79	Slight
0.21 - 0.50	Moderate
<0.17	Severe

The summary of bioassessment scores in Table 5 shows that the aquatic community of the GM Powertrain receiving stream was "moderately" impaired. Its bioassessment score was less than both the unnamed tributary (Site 2) and the reference stream (Sites 3 and 4).

The unnamed tributary had an aquatic community which was "slightly" impaired. Its bioassessment score was higher than the GM Powertrain receiving stream score but less than the reference stream score.

Table 1.

Macroinvertebrate Sampling Results

Unnamed GM Powertrain Receiving Stream
Site 1
December 4, 1992

Bedrock Sample

Diptera	
Simuliidae	1
Chironomidae	
Psectrocladius psilopterus	1
Chironomus riparius group	1
Polypedilum illinoense	1
Trichoptera	
Hydropsyche betteni	88
Oligochaeta (Tubificidae)	8

CPOM (Coarse Particulate Organic Matter) Sample

Shredders	0
Non-shredders (Chironomidae, Hydropsychidae, Tubificidae)	8

BIOMETRICS

Total Number of Genera - 6
Total Number of EPT (Ephemeroptera, Plecoptera, Trichoptera) Genera - 1
Ratio of Scrapers/Filtering Collectors - 0.0
Ratio of EPT Abundance/Chironomids - 29.3
Ratio of Shredders/Total - 0.0
Percent Contribution of Dominant Taxon - 88%
Community Loss Index - 1.7
Hilsenhoff Biotic Index - 6.2

Table 2.

Macroinvertebrate Sampling Results

Tributary to Unnamed GM Powertrain Receiving Stream
Site 2
December 4, 1992

Bedrock Sample

Diptera	
Tipulidae	7
Chironomidae	
Parametriocnemus sp.	1
Psectrocladius psilopterus	4
Trichoptera	
Hydropsyche betteni	5
Cheumatopsyche sp.	3
Isopoda (*Lirceus* sp.)	1
Mollusca	
Sphaeridae	1
Physa sp.	1

* Only 23 organisms in sample, even after intensive effort

CPOM Sample

Shredders (Isopoda and Tipulidae)	19
Non-shredders (Chironomidae & Tubificidae)	3

BIOMETRICS

Total Number of Genera - 8
Total Number of EPT Genera - 2
Ratio of Scrapers/Filtering Collectors - 0.0
Ratio of EPT Abundance/Chironomids - 1.6
Ratio of Shredders/Total - 0.86
Percent Contribution of Dominant Taxon - 30%
Community Loss Index - 1.3
Hilsenhoff Biotic Index - 5.4

Table 3.

Macroinvertebrate Sampling Results

Willow Creek (Reference Stream)
Site 3
December 4, 1992

Bedrock Sample

Diptera	
Simuliidae	3
Chironomidae	
Cardiocladius sp.	21
Diplocladius sp.	6
Psectrocladius psilopterus	4
Cricotopus sylvestris	4
Orthocladius obumbratus	2
Ablabesmyia sp.	3
Trichoptera	
Hydropsyche betteni	24
Cheumatopsyche sp.	10
Cyrnellus fraternus	10
Ephemeroptera	
Stenonema vicarium	11
Stenacron interpunctatum	1
Plecoptera (*Allocapnia* sp.)	1

CPOM Sample

Shredders (Filipalpia and Tipulidae)	7
Non-shredders	108

BIOMETRICS

Total Number of Genera - 13
Total Number of EPT Genera - 6
Ratio of Scrapers/Filtering Collectors - 0.26
Ratio of EPT Abundance/Chironomids - 1.4
Ratio of Shredders/Total - 0.06
Percent Contribution of Dominant Taxon - 24%
Community Loss Index - 0.0
Hilsenhoff Biotic Index - 5.8

Table 4.
Macroinvertebrate Sampling Results

DUPLICATE
Willow Creek (Reference Stream)
Site 4
December 4, 1992

Bedrock Sample

Diptera	
Simuliidae	8
Chironomidae	
Cardiocladius sp.	31
Diplocladius sp.	19
Cricotopus sylvestris	6
Orthocladius obumbratus	1
Trichoptera	
Hydropsyche betteni	14
Cheumatopsyche sp.	5
Cyrnellus fraternus	2
Ephemeroptera	
Stenonema vicarium	9
Plecoptera	
Allocapnia sp.	1
Isoperla sp.	1
Coleoptera (Elmid larvae)	2
Amphipoda	1

CPOM Sample (not duplicated)

Shredders (Filipalpia and Tipulidae)	7
Non-shredders	108

BIOMETRICS

Total Number of Genera - 13
Total Number of EPT Genera - 6
Ratio of Scrapers/Filtering Collectors - 0.38
Ratio of EPT Abundance/Chironomids - 0.56
Ratio of Shredders/Total - 0.06
Percent Contribution of Dominant Taxon - 31%
Community Loss Index - 0.0
Hilsenhoff Biotic Index - 6.0

Table 5.

SUMMARY OF MACROINVERTEBRATE BIOMETRICS AND SCORING FROM EACH SITE
(Scores are based on comparison to reference site 3)

	BIOMETRICS			
Site No.	1	2	3	4
No. of Genera	6	8	13	13
EPT Genera	1	2	6	6
Scrapers/Filterers Ratio	0.0	0.0	0.26	0.38
EPT/Chironomid Abundance	29.3	1.6	1.4	0.56
Percent Shredders	0.0	86	6	6
Percent Dominant Taxon	88	30	24	31
Community Loss Index	1.7	1.3	0.0	0.0
Hilsenhoff Biotic Index	6.2	5.4	5.8	6.0

	SCORING			
Site No.	1	2	3	4
No. of Genera	2	4	6	6
EPT Genera	0	0	6	6
Scrapers/Filterers Ratio	0	0	6	6
EPT/Chironomid Abundance	6	6	6	2
Percent Shredders	0	6	6	6
Percent Dominant Taxon	0	2	4	2
Community Loss Index	2	4	6	6
Hilsenhoff Biotic Index	6	6	6	6
Site Score	16	28	46	40
Percent of Reference	35	61	100	87
Impairment	moderate	slight	none	none

1 = GM Receiving Stream 2 = Tributary 3,4 = Reference Sites

VI. Possible Causes of Impairment

By examining the composition of an "impaired" benthic community and applying a knowledge of how animals are affected by different forms of stress, it is sometimes possible to determine causes of impairment. Causes of impairment in the GM receiving stream (site 1) and its unnamed tributary (site 2) are discussed separately below.

The benthic community downstream from GM Powertrain outfall 002 (site 1) did not appear to be stressed by toxic substances. Toxicity-related effects are usually manifested by a decline in total biomass and the dominance of the community by one or two toxicity-tolerant species. Although total diversity was low, benthic animals in the GM receiving stream were very abundant and were dominated by caddisfly larvae, a group usually intolerant to most forms of toxic pollution.

Likewise, site 1 did not appear to be affected by low dissolved oxygen caused by "organic" pollution. The Hilsenhoff Biotic Index (HBI), which is a highly sensitive indicator of stress caused by low dissolved oxygen, was similar at all sites (see Table 5).

There is strong evidence that much of the impairment observed at Site 1 (the GM receiving stream) was due to runoff from coal spoils in the watershed. Wangsness (USGS Open File Report 82-566) observed that most EPT genera (environmentally sensitive aquatic insects) were eliminated from streams draining mined areas in Indiana. Resistant forms included certain hydropsychid caddisflies, midge larvae, and blackfly larvae, all of which were common or dominant at Site 1. Similar results were observed in mine-impacted streams in Pennsylvania (Letterman & Mitsch, 1978. Environmental Pollution 17:53-73).

The unnamed tributary (site 2) had a somewhat different benthic community. Although total diversity was similar to that observed at site 1, biomass was very low (only 23 animals were collected from the stream despite extensive sampling). Orange-colored (ferric hydroxide derived) sediments typical of streams draining previously mined areas were common in this stream. These loose, easily sloughed sediments appeared to be clogging much of the available benthic habitat and probably contributed to the impairment observed in this tributary.

VII. Comparison to Previous Studies

The aquatic communities of the GM Powertrain receiving stream and its unnamed tributary were studied by Michael E. Bender of the Virginia Institute of Marine Science in 1976 and 1978. Bender's studies were conducted in November and December, which is the same sampling season used in the present study. His sampling techniques (Surber samples from riffle areas) differed somewhat from those used here, but a fairly close comparison between the two studies is still possible. This comparison would allow a rough determination of whether the aquatic communities of the two streams have improved or deteriorated over the years.

Bender's samples from the GM Powertrain receiving stream (site 1 in this study) showed that the stream supported virtually no benthic life downstream from the GM wastewater outfall. Only a few scattered oligochaetes (highly pollution-tolerant aquatic worms) were present in the stream both years. Bender attributed the paucity of benthos in this stream as an indication of "toxic pollutants" (primarily zinc).

Likewise, samples from 1976 and 1978 in the unnamed tributary (site 2 in this study) contained few types of aquatic organisms. These samples contained numerous oligochaetes (normally indicative of "organic" or sewage-related pollution) and one or two other genera of midge larvae or snails. Bender attributed the high benthic density but generally low diversity to an inadequately treated wastewater discharge from the Tilton Wastewater Treatment Plant, immediately upstream from the sampling site.

The benthic community of the GM Powertrain receiving stream (site 1) appears to have improved tremendously since the late 1970's. The number of genera present has increased from 1 to at least 6 and the dominant animals are generally pollution-sensitive caddisflies rather than pollution-tolerant aquatic worms. The density of organisms has probably increased significantly as well (only 1 or 2 animals were present per square foot previously, compared to the 1992 study in which at least a hundred animals could easily be collected in a square foot area).

Likewise, the benthic community of the unnamed tributary (site 2) appears to be somewhat improved since the 1970's. The number of genera present has increased from 2 or 3 to at least 8 and relatively intolerant caddisfly larvae have replaced tolerant aquatic worms as the dominant life form. Although the 1992 samples were not strictly quantitative, the density of animals appears to be much less now than previously. This change could be a reflection of decreased organic inputs to the stream (the Tilton Wastewater Treatment Plant no longer discharges to this tributary).

VIII. Evaluation of Potential Fluoride Effects on the GM Powertrain Receiving Stream

Because of the interference caused by coal spoils-related impairment, the effect of fluoride in GM Powertrain wastewater on the aquatic communities of these streams is difficult to determine. A toxicity literature search (see Review of Fluoride Toxicity Data report, submitted separately) revealed no toxicity data for fluoride effects on any of the benthic species collected in this study. Therefore, their relative tolerances to fluoride are not directly known.

However, the Review of Fluoride Toxicity Data showed that even the most sensitive species tested for fluoride toxicity, as reported in the scientific literature, showed no adverse effects at 10 mg/l in hard water. Except for one period during early 1992 when the company used a coke substitute in the foundry process, the GM 002 wastewater never exceeded 10 mg/l fluoride and its hardness is consistently higher than 300 mg/l. These observations lend additional evidence that fluoride in the GM Powertrain wastewater are not causing instream toxicity.

In addition, the benthic community of the stream was not typical of those affected by most toxic substances. Many studies by numerous aquatic scientists have consistently shown that toxics-affected streams are characterized by both low diversity and low numbers of animals (a bibliography of several such studies is included in the Appendix). Although its overall diversity was low, the GM Powertrain receiving stream supported a very abundant benthic community, dominated by what most aquatic biologists regard as pollution-intolerant hydropsychid caddisfly larvae. If fluoride toxicity was causing impairment in this stream, these benthic animals would almost certainly be much reduced in density below what was observed here.

VII. Conclusions

The aquatic habitat value of the GM Powertrain receiving stream and an unnamed tributary to this stream were determined to be "good" using a standard EPA evaluation procedure. Although not able to support a diverse fishery because of its steep gradient and numerous waterfalls and debris dams, the GM receiving stream had adequate habitat to support a diverse benthic community.

Despite their good aquatic habitats, the GM Powertrain receiving stream and its unnamed tributary both had impaired benthic macroinvertebrate communities when compared to a relatively pristine reference stream nearby.

Much of the impairment observed in these two study streams was probably due to runoff from coal spoils in the watershed. Stress from total dissolved solids (chloride, sulfate, calcium, magnesium, sodium) derived from this runoff probably contributed to the impairment. Unless this runoff is controlled, it is unlikely that the impairment observed in the two study streams will improve significantly.

Fluoride concentrations in GM outfall 002 probably have little or no toxic effect on the aquatic community of the GM receiving stream or on the Vermilion River downstream from the outfall. This conclusion is based on both laboratory studies reported in the scientific literature (showing that 10 mg/l in hard water is not chronically toxic to the most sensitive animals tested) and this field study (showing that benthic life in the stream was not typical of toxics-affected streams).

The aquatic communities of the GM receiving stream and its tributary appear to have improved significantly since the 1970's. These changes are probably related to improvements in the GM treatment process and the discontinuation of discharge from the Tilton Wastewater Treatment Plant.

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The following bibliography is a partial listing of studies which have reported on characteristics of toxics-affected stream communities and which have shown reduced abundance or biomass of benthos as a characteristic of these streams (grouped by different toxic input groups).

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HEAVY METALS

Hynes, H.B.N. 1960. The biology of polluted waters. Liverpool University Press, Liverpool, England. 202 p. (lists and summarizes numerous studies of metals-impacted streams).

UNIDENTIFIED TOXIC SUBSTANCES

Klemm, D.J. et al. 1990. Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. USEPA Office of Research and Development. EPA/600/4-90/030.

Review of Fluoride Toxicity Data

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Review of Fluoride Toxicity Data

OBJECTIVE

The objective of this review was to determine levels of fluoride which would not cause aquatic toxicity to freshwater communities in North America.

METHODS

We assembled all available information on the toxicity of fluoride to aquatic animals. Much of this information was present in the USEPA database AQUIRE, which is maintained and updated by the EPA Environmental Research Laboratory at Duluth, Minnesota. The information was edited to include only the following:

Data on North American freshwater species
Data from laboratory toxicity tests
  48 or 96-hr acute tests
  partial life-cycle or early life stage chronic tests
Data with review codes of 1 or 2 (the data had been subjected to most EPA quality assurance procedures to confirm its precision, accuracy, and repeatability.

Other information which was not in AQUIRE (including data available after 1990) was also included in this literature search. Copies of all available reports are attached in an Appendix.

Because much of the scientific literature on fluoride suggests that there is a relationship between fluoride toxicity and hardness, the hardness values of water used in the toxicity tests was reported whenever possible.

RESULTS OF DATABASE SEARCH

The literature search revealed acceptable information on the aquatic toxicity of fluoride for eight North American freshwater animal species. Six of these species were fish. The available database was not extensive enough to calculate water quality criteria using USEPA's method published in 50 F.R. 30784, July 29, 1985. Specifically, data were lacking for a benthic crustacean and an aquatic insect. Acute to chronic ratios were available for only two of the three required groups.

Although a true water quality criterion for fluoride could not be calculated using USEPA's technique, an estimated criterion based on the technique is still possible. The purpose of such an estimated criterion is to determine a "threshold" level of fluoride, below which aquatic toxicity to most organisms in North American freshwaters should not occur.

SUMMARY OF ACUTE TOXICITY EFFECTS

The lowest acute effect observed for fluoride toxicity to a freshwater animal species was 51 mg/l to rainbow trout, while mosquitofish (Gambusia affinis) appeared to be the most tolerant species tested. The range of LC50 values available for all species in the database was relatively small (minimum 51 mg/l, maximum 418 mg/l). This small range of toxicity values means that a criterion protective of the most sensitive species in the database would probably protect almost all species potentially exposed to fluoride.

SUMMARY OF CHRONIC TOXICITY EFFECTS

Chronic toxicity information was available for two species. The lowest reported chronic value to a freshwater animal species was 2.7 mg/l to rainbow trout in very soft water. However, the chronic value in water with hardness greater than 100 mg/l was 25 mg/l for Daphnia magna.

Acute to Chronic Ratios for these two species ranged from 9.0 to 18.9 for tests done under similar conditions. The geometric mean of these A/C ratios was 13.4.

SUMMARY OF HARDNESS-RELATED EFFECTS ON FLUORIDE TOXICITY

Four species have been used in aquatic toxicity tests in which hardness values of the test water were significantly different in two or more tests. The following summary shows these effects:

	LC50	hardness
Rainbow trout	51	17
	128	49
	140	182
	193	385
Daphnia magna	154	72
	227	100
	279	169
Fathead minnow	180	92
	205	256
Threespine stickleback	340	78
	380	146
	460	300

For each of these species, toxicity decreased with increasing hardness. The inverse relationship between hardness and fluoride toxicity may be due to changes in fluoride speciation occuring in high-hardness waters. Several authors have remarked on the chemistry of fluoride in the presence of relatively high calcium concentrations (high-hardness waters).

For example, Smith et al. (Reference 8) observed that "combinations of high fluoride and moderate to high hardness caused rapid precipitation of finely divided solid, which spectorgraphic analysis indicated to consist of calcium and magnesium salts". In their tests with water of an initial hardness of 256 mg/l, the hardness dropped to 12 mg/l within a few hours after the addition of 400 mg/l fluoride (as sodium fluoride). Vallin (reference 16) noted a formation of calcium fluoride precipitate in his fluoride tests with hardness values of 320 mg/l. Apparently, fluoride combines easily with calcium in high-hardness water to form the relatively insoluble compound calcium fluoride. Once out of solution, the fluoride precipitate is in a form which is not readily available as a toxicant.

The relationship between toxicity and hardness can be expressed mathematically using the technique employed by EPA in the Gold Book (Water Quality Criteria for Water 1986, EPA 440/5-86-001). All data are normalized and a least squares regression on the normalized data is performed. The technique produces a pooled slope of the regression, by which predicted toxicity at any given hardness value may be calculated for each species.

Slopes of the regression range from 0.1524 for fathead minnows to 1.1874 for Daphnia magna. When all the available data from all four species are used in the analysis, the pooled slope of the toxicity-hardness regression is 0.2288. EPA uses the following equation to predict acute toxicity effects at various hardness values for each species:

$$Y = \ln W - V(\ln X - \ln Z)$$

where Y = predicted LC50
W = geometric mean of the LC50 values available
V = pooled slope
X = geometric mean of all hardness values available
Z = selected hardness value

The lowest predicted acute LC50 value (for the most sensitive species, brook trout) would vary with hardness as follows:

	LC50
hardness 100	119
hardness 200	139
hardness 300	153
hardness 400	163

Although a chronic slope cannot be calculated because of limited data, tests with brook trout indicate that chronic toxicity of *fluoride is* also inversely related to hardness. This result is consistent with EPA's analysis of hardness effects on other toxicants. For example, six of the seven toxicants studied by EPA for hardness related effects showed that hardness affects both acute and chronic toxicity (EPA Gold Book, 1985).

Given this inverse relationship between hardness and toxicity, it appears that acute fluoride toxicity in waters where hardness exceeds 300 mg/l should not be observed to even the most sensitive species in the database until the fluoride concentration exceeds 150 mg/l.

Assuming that chronic fluoride toxicity is similarly related to hardness and that an acute to chronic ratio of 13.4 (the geometric mean of three values reported for two species) is representative, fluoride in waters where hardness exceeds 300 mg/l should not cause chronic toxicity until concentrations exceed 11 mg/l (the predicted acute toxicity to brook trout divided by the A/C ratio).

These concentrations appear reasonably protective because an array of field and laboratory data show no toxicity effects below these values. For example, a thriving population of brown trout (closely related to brook trout) exist in the Firehole River of Montana, where fluoride concentrations are as high as 14 mg/l (reference 12). Another field study done in Colorado showed that benthic community in a "softwater" Colorado stream showed no reduction in diversity where fluoride averaged 3.5 mg/l (reference 14). In laboratory studies, 11 mg/l fluoride is less than the lowest chronic value observed for any animal in tests where hardness exceeded 100 mg/l.

In addition, Daphnia magna are known to be among the most sensitive of all freshwater animals to most toxicants. The observation that 11 mg/l fluoride has not adversely affected this sensitive test animal adds additional confidence to the value. Finally, the most sensitive animal in the fluoride database (brook trout) is found only in cool streams of northern North America. It would not be a potential resident of streams where water temperature regularly exceeds 20 degrees Centigrade. Therefore, a criterion which is based on protection of this species, even where it is not a potential resident, would appear to be even more restrictive than necessary to protect the rest of the aquatic community.

AQUATIC TOXICITY INFORMATION FOR FLUORIDE

Acute Toxicity

Rank	Species	48 or 96 -hr LC50 mg/l	hardness mg/l	reference
1	Rainbow trout	51	17	2
	Rainbow trout	128	49	2
	Rainbow trout	140	182	2
	Rainbow trout	193	385	2
2	Carp	75-91	10	1
3	Daphnia magna	154	72	4
	Daphnia magna	227	100	5
	Daphnia magna	279	169	19
4	Philodina acuticornus (rotifer)	158		6
5	Bluegill	>239		7
6	Fathead minnow	315	20-48	8
	Fathead minnow	180	92	8
	Fathead minnow	205	256	8
7	Threespine stickleback	460	300	8
	Threespine stickleback	380	146	8
	Threespine stickleback	340	78	8
8	Mosquitofish	418		9

Chronic Toxicity

Rank	Species	Chronic Value mg/l	Hardness mg/l	Reference
1	Rainbow trout	2.7	10	1
		>100	320	16
2	Daphnia magna	25	230	18
		31	169	19

Acute to Chronic Ratios

Species	Acute Value	Chronic Value	A/C Ratio
Rainbow trout	51	2.7	18.9
Daphnia magna	<352	25	<14.1
	279	31	9.0

The geometric mean of these three A/C Ratios is 13.4

Other Data

Species	Effect	Concentration mg/l	Reference
Green algae Scenedesmus subspicatus	4-day EC50	900	10
Green algae Selenastrum capricornutum	4-day EC50	122	7
Leopard frog Rana pipiens	reduced mobility heart enlargement	>50	11
Brown trout	healthy specimens in Firehole River	14	12
Goldfish	mortality seen after 4 days	100	13
Benthos in Colorado softwater stream	no reduction in diversity	3.5	14
Ceriodaphnia dubia	48-hr LC50	120-340	15
Rainbow trout	100-hr LC50 in water with no "hardness"	6-22	17

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RESULTS OF THE HARMFUL EFFECTS OF WATER POLLUTANTS TO *DAPHNIA MAGNA* IN THE 21 DAY REPRODUCTION TEST

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Abstract—Investigations were carried out in order to determine the "no observed effect concentration" (NOEC) of 73 environmentally relevant substances in the 21 d *Daphnia* reproduction test. The test was conducted in line with the provisional procedure proposed by the Federal Environmental Agency (Umweltbundesamt) (as of 1 January 1984). *Daphnia magna* Straus was used as the test organism. Evaluation parameters for fixing the NOEC were the mortality of the parent animals, the reproduction rate and the appearance of the first offspring during the test period. The concentrations of substances in the test preparations were chemically quantified. The NOEC values obtained for ethyl parathion, bis(tri-*n*-butyltin)oxide and the active ion Cd (II) were in the concentration range 1 ng l^{-1}–1 $\mu g\,l^{-1}$, for 13 tested substances in the range 1 $\mu g\,l^{-1}$–<0.1 mg l^{-1} and for 23 substances in the range 0.1–<1 mg l^{-1}. In further evaluation of the results, it could be calculated that the substance concentration of the NOEC for three substances was more than 3 logs, in the case of 13 substances more than 2 logs, for 10 substances 50–90 times, for 24 substances 10–40 times and for 14 substances 5–9 times lower than the 24 h EC_{50} of the corresponding substance.

Key words—*Daphnia magna*, 21 d reproduction test, NOEC, water pollutants

INTRODUCTION AND OUTLINE OF PROBLEM

Toxicological investigations were conducted into the potentially harmful effects of inorganic and organic substances in water on *Daphnia magna*, in particular on its reproduction and survival rate during the test period of 21 days. The test was conducted in accordance with the "Provisional Procedure: extended toxicology test with *Daphnia magna* (determination of NOEC for reproduction rate, mortality and the time of the first appearance of offspring; 21 d)" as of 1 January 1984, issued as the "Recommendation of the Federal Environmental Agency on the Performance of Testing according to Section 5, para 1 No. 3 of the Regulation on Application Documents and Evidence under the Chemicals Act" (Federal Environmental Agency). The investigation covered potential pollutants which have various chemical properties and/or belong to different groups, i.e.

water soluble,
volatile,
poorly soluble substances.

The concentrations of substances in the test preparations were quantified chemically. In order to obtain comparable results on the tested substances, the test procedure was retained during the investigation period.

METHODS

Test organism—Daphnia magna

The *Daphnia magna* strain (IRCHA strain) has been maintained in accordance with the procedure practised since 1978. In each case, 20–30 specimens were placed in forty 2-l. beakers which had been filled with at least 1.6 l. Berlin tap water. They provided 24 h-old animals when the offspring were removed daily from the cultures.

For all *Daphnia* strain cultures, temperature-controlled, dechlorinated and oxygen-saturated tap water (German hardness 16°, pH value 7.6–7.7) was used which had been left to stand for 24 h. Before collecting the water, the tap was turned on fully and left to run for at least 1 h.

All beakers were covered with watch glasses and placed on a white supporting surface. Feeding with dry algae of the *Scenedesmus* genus took place daily. Nine g of feed were suspended in 1000 ml tap water and 2 ml of the suspension were added to each beaker.

The temperature of the culture area was regulated thermostatically at 20°C. Under exclusion of daylight, the area was lit by fluorescent lamps (Philips TL 65/33W) for 9 h between 7 a.m. and 4 p.m.

On Monday and Thursday of each week the tap water in all beakers was renewed as were the beakers themselves on Mondays. On Mondays, the offspring which had appeared between Thursday or Friday and Monday were concentrated using the 0.315 mm DIN sieve and separated according to size using the 0.630 mm DIN sieve. *Daphnia* in the different size categories were used separately for further cultivation.

In order to obtain 24 h-old animals on the potential preparation days in a 21 d test series—Wednesdays or Fridays—it was necessary to remove the offspring from

the cultivation beakers on Tuesday and/or Thursday. The daphnids which were at most 24 h old were removed by pipette and concentrated on a 0.25 mm DIN sieve, placed in as small an amount of dilution water as possible and used as test organisms.

Dilution water

In the interests of national and international standardization, an artificial medium (synthetic fresh water) (DIN—German Institute of Standardization, 1982a, b) of the following composition was used in the test and control preparations:

11.76 g $CaCl_2 \cdot 2H_2O$ (A.R.)/1 litre deionized water
4.93 g $MgSO_4 \cdot 7H_2O$ (A.R.)/1 litre deionized water
2.59 g $NaHCO_3$ (A.R.)/1 litre deionized water
0.23 g KCl (A.R.)/1 litre deionized water.

Twenty-five millilitres of each solution was pipetted into a graduated flask and completed to 1 litre with deionized water. The amount of calcium and magnesium ions in this solution was 2.5 mmol l^{-1}. The molar relationship of sodium to potassium ions was 10:1. This water was aerated up to the water saturation level and the pH value was measured (8.0 ± 0.2). When using deionized water with a conductivity of $< 1\ \mu S\ cm^{-1}$, the dilution water was diluted with 10% tap water.

Test procedure

Before preparing the dilution series, the substances to be tested were fully dissolved (both quantitatively and optically) in dilution water using magnetic stirrers (stock solution). In the case of substances of low water solubility, efforts were made to dissolve them by means of up to 24 h stirring. Solutions which were not optically clear after dissolution, were filtered over fibre-glass filters and the filtrate was quantified chemically. Pure ethanol in a concentration of 5 μl 1000 ml^{-1} dilution water was used as a solubilizer in the case of two substances which only dissolved with great difficulty [bis(tri-*n*-butyltin)oxide and ethyl parathion]. The preparation and concentration of the stock solution for each tested substance are given in Table 4.

From the stock solution of the substance to be tested, graduated dilutions with dilution water were produced in the concentration range in which effects were to be expected in accordance with the results from the acute 24 h *Daphnia* test and a preliminary 3 d *Daphnia* test (same conditions as in the 21 d reproduction test). The number of dilution steps depended on the effect of the respective substance. Efforts were made to determine both the NOEC and the concentration at which a lethal effect could be observed on all the parent animals. The dilution steps corresponded to a ratio of 1:2. If it was necessary to use a concentration range of the substance of 3–4 logs in the test, then the dilution series was set up with a ratio of $1:\sqrt{10}$. 400 ml beakers with 250 ml useful capacity were used as test vessels. In the case of volatile or strongly smelling substances, 250 ml wide-neck bottles with ground-glass stoppers were used.

Four parallel test vessels per concentration level and the controls comprising at least four vessels, were filled with 24 h-old *Daphnia*—1 animal/50 ml—and this meant 20 test animals per concentration level.

The semi-static procedure adopted meant that the parent animals in the test and control vessels had to be pipetted 3 times a week (Mondays, Wednesdays and Fridays) into freshly prepared test and control media—in each case at the corresponding concentration level. During this process, dead parent animals or those incapable of swimming were removed. The offspring were counted and the total number for each test vessel was recorded. Then, the pH value and the oxygen concentration were measured in two test vessels per concentration level.

The test and control preparations were observed daily in order *inter alia*, to record the day on which the first offspring appeared. In the case of open test vessels (beakers covered with watch glasses), feeding was carried out at the same time. In the case of closed test vessels (bottles closed with ground-glass stoppers), feeding could only take place on the days on which the parent animals were transferred. The amount of feed given at that time was 2–3 times higher than the daily amount given in the open vessels. Tetramin-Hauptfutter (fish feed) and activated sludge were used as feeds. This led to an overall COD of 15–20 mg l^{-1} as the daily feed amount.

The test culture area was protected from daylight and lit from 7 a.m. to 4 p.m. with fluorescent lamps—Philips TL 40/25W. Unlike the procedure proposal, the temperature of the test area was set thermostatically at 25 ± 1°C in order to be sure of meeting the stipulated quality criteria.

For reasons of practicability, the chemical determination of the substance concentrations laid down in the test guideline was modified as follows. Samples were taken twice from selected concentration levels of the test series during the test period and analysed chemically: the first sampling took place on one of the transfer days before the 7th day, i.e. in the period during which no offspring appeared; the second sampling took place between the 16th and 21st day. For the corresponding dilution levels, the following parameters were determined:

the concentrations of the initial preparations in order to check the solution behaviour and the dilution steps;
the concentrations in the test and blank preparations (no test organisms or feed) after an interval of 48/72 h in order to determine the fate of the substance.

STATISTICAL EVALUATION OF THE RESULTS

The parameters for fixing the NOEC of the substance were the mortality of the parent animals, the reproduction rate and the appearance of the first offspring during the test period. The Student's *t*-test and the U-test were the statistical methods used to calculate the first two parameters (Sachs, 1969). The U-test did not require the normal distribution of the test results and was therefore more suitable for evaluating the 21 d *Daphnia* reproduction test. The highest concentration level, which did not differ (NOEC) from the control, and the lowest, which did differ from the control in respect of mortality of the parent animals and of the reproduction rate, could be ascertained from the calculations. Moreover, in order to determine the NOEC, observations and evaluations were also made of the appearance of the first offspring in the test preparations in comparison with the control preparations. Primarily, the results were expressed with reference to the nominal concentration. If, however, the chemical analysis showed a loss of the tested substance of more than 20%, then the lowest analysed concentration (minimum value) obtained during the test was also given for the NOEC. The aim was to counteract the customary discrepancies between interpretation and the reproducibility of chemical measured data which move within a range of variance.

PRESENTATION AND ASSESSMENT OF THE RESULTS

In order to facilitate comparison of the results, the following data were included in Table 1 on the substances listed according to substance group:

Table 1. Comparative presentation of the results of the harmful effects of water pollutants in the acute and in the 21 d *Daphnia* reproduction test

Table 1. Comparative presentation of the results of the harmful effects of water pollutants in the acute and in the 21 d *Daphnia* reproduction test

Tested substances	Acute *Daphnia* test: 24 h EC_0 Nominal value ($mg\,l^{-1}$)	Acute *Daphnia* test: 24 h EC_{50} ($mg\,l^{-1}$)	21 d *Daphnia* reproduction test: 21 d NOEC Nominal value ($mg\,l^{-1}$)	21 d NOEC Minimum value ($mg\,l^{-1}$)	Most sensitive parameter	Tested concentration range	Dilution ratio	Test vessel*
Inorganic compounds								
Cadmium chloride: Cd^{2+}	0.94	1.9	0.0006		B	0.0006–1.94 $\mu g\,l^{-1}$	1:$\sqrt{10}$	
$CdCl_2$	1.5	3.0	0.001			0.001–3.16 $\mu g\,l^{-1}$		
Potassium dichromate: Cr^{6+}	0.11	0.35	0.018		A and B	4.6–142 $\mu g\,l^{-1}$	1:2	
$K_2Cr_2O_7$	0.30	0.93	0.050			13–400 $\mu g\,l^{-1}$		
Chromium chloride: Cr^{3+}	11	22	0.70		B	0.08–11 $mg\,l^{-1}$	1:2	
$CrCl_3 6H_2O$	55	111	3.4			0.43–55 $mg\,l^{-1}$		
Sodium fluoride: F^-	231	352	14		B	0.15–453 $mg\,l^{-1}$	1:$\sqrt{10}$	
NaF	510	777	32			0.32–1000 $mg\,l^{-1}$		
Sodium bromide: Br^-	5171	7219	91		B and C	2.3–8871 $mg\,l^{-1}$	1:2.5	
NaBr	6671	9313	117			3–11,444 $mg\,l^{-1}$		
Nickel acetate: Ni^{2+}	5.7	21	0.090		A and B	0.09–23.6 $mg\,l^{-1}$	1:2	
$Ni(CH_3COO)_2 4H_2O$	24	87	0.40			0.4–100 $mg\,l^{-1}$		
Organometallic compounds								
bis(tri-*n*-butyltin)oxide	0.010	0.030	0.00016		B	0.08–10 $\mu g\,l^{-1}$	1:2	‡
Organophosphorus compounds								
Phosphoric acid tributyl ester	9.3	35	1.3		B	0.01–10.0 $mg\,l^{-1}$	1:2	
Ethyl parathion	0.0007	0.002	0.000002		A, B and C	0.5–500 $ng\,l^{-1}$	1:2	
Halogenated alkanes								
Chloroform	48	79	13	6.3	A, B and C	1.57–200 $mg\,l^{-1}$	1:2	‡
Esters								
Ethyl acetate	1822	2306	12	2.4	A and B	1.5–375 $mg\,l^{-1}$	1:2	
Propionic acid methyl ester	121	516	6.3	3.2	A and C	1.6–200 $mg\,l^{-1}$	1:2	‡
Propionic acid ethyl ester	173	286	6.3	1.3	A	0.8–50 $mg\,l^{-1}$	1:2	‡
Phthalic acid diethyl ester	20	86	13	3.8	A, B and C	0.4–100 $mg\,l^{-1}$	1:2	
Phthalic acid dibutyl ester	8.9	17	1.0	0.5	A	0.06–8 $mg\,l^{-1}$	1:2	
Phthalic acid diallyl ester	11	26	3.2		A, B and C	0.025–25 $mg\,l^{-1}$	1:2	
Alcohols								
Isobutanol	837	1250	20	4.0	B	10–1250 $mg\,l^{-1}$	1:2	
Isooctanol	77	115	2.3	1.6	B and C	0.6–150 $mg\,l^{-1}$	1:2	
1-Octanol	19	26	1.6	1.0	C	0.4–50 $mg\,l^{-1}$	1:2	
1,3-Dichloro-2-propanol	558	983	16	10.4	B	8–1000 $mg\,l^{-1}$	1:2	
2,3-Dibromo-1-propanol	383	536	16	9.6	B and C	4–500 $mg\,l^{-1}$	1:2	
Monocarboxylic acids					A, B and C	0.032–100 $mg\,l^{-1}$	1:$\sqrt{10}$	
Monochloroacetic acid	85	96	32		A	0.032–100 $mg\,l^{-1}$	1:$\sqrt{10}$	
Monobromoacetic acid	34	65	3.2	1.6				

continued overleaf

Table 1—*continued*

Tested substances	Acute *Daphnia* test: 24 h EC_0 Nominal value (mg l^{-1})	Acute *Daphnia* test: 24 h EC_{50} Nominal value (mg l^{-1})	21 d *Daphnia* reproduction test: 21 d NOEC Nominal value (mg l^{-1})	21 d NOEC Minimum value (mg l^{-1})	Most sensitive parameter	Tested concentration range	Dilution ratio	Test vessel*
Aldehydes								
Chloroacetaldehyde (50% sol.)	6.6	15.0	5.0		A, B and C	0.08–10 mg l^{-1}	1:2	
Salicylaldehyde	0.90	3.1	0.63	0.38	B	0.04–5 mg l^{-1}	1:2	
Ketones								
Methyl isobutyl ketone	930	3682	78	7.8–39	B	20–2500 mg l^{-1}	1:2	
Amines								
Ethylenediamine	3.5	14	0.16		B	0.08–5 mg l^{-1}	1:2	‡
Triethanolamine	1530	2038	16		A	8–1000 mg l^{-1}	1:2	‡
Aniline	0.10	0.90	0.010	0.004	B	0.1–316 μg l^{-1}	1:$\sqrt{10}$	‡
2,3-Dimethylaniline	1.6	10	0.16	0.1	B	0.02–2.5 mg l^{-1}	1:2	‡
3,4-Dimethylaniline	0.20	2.9	0.016	0.01	B	0.016–2.0 mg l^{-1}	1:2	‡
o-Tolidine	1.5	3.2	0.16		B	0.04–5.0 mg l^{-1}	1:2	‡
Chloroamine T	2.7	4.8	1.3		B and C	0.08–20 mg l^{-1}	1:2	‡
Niclosamide†	0.058	0.16	0.020		A and B	0.005–0.6 mg l^{-1}	1:2	‡
Aromatic nitro compounds								
Nitrobenzene	19	60	13	2.6	B	1.6–200 mg l^{-1}	1:2	
2-Nitroanisole	37	65	13		A, B and C	0.8–100 mg l^{-1}	1:2	
4-Nitroanisole	9.3	15	3.2		A and B	0.8–25 mg l^{-1}	1:2	
4-Nitrophenol	3.0	8.0	1.3		A and B	0.08–10 mg l^{-1}	1:2	
2,4-Dinitrophenol	4.8	7.0	2.0		B and C	0.063–8 mg l^{-1}	1:2	
2,4,6-Trinitrophenol (picric acid)	31	85	5.0		A and B	0.63–80 mg l^{-1}	1:2	
2,6-Dinitrotoluene (10% water)	11	20	0.16	0.060	A and B	0.16–20 mg l^{-1}	1:2	
2,4-Dinitrotoluene (10% water)	13	38	0.040	0.020	B	0.02–2.5 mg l^{-1}	1:2	‡
2-Nitro-*p*-cresol	19	52	2.3		B and C	1.13–72 mg l^{-1}	1:2	
4,6-Dinitro-*o*-cresol	1.5	2.3	1.3		B	0.02–2.5 mg l^{-1}	1:2	
Phenols								
2,4,6-Trimethylphenol	1.8	3.4	0.10		B and C	0.3–3162 mg l^{-1}	1:$\sqrt{10}$	
3,5-Dimethoxyphenol	1.0	14	0.10	0.04	C	0.003–10 mg l^{-1}	1:$\sqrt{10}$	
2-Amino-4-methylphenol	0.49	3.3	0.25		A	0.03–4 mg l^{-1}	1:2	
p-Cresol	2.5	4.9	1.0		A	0.003–10 mg l^{-1}	1:$\sqrt{10}$	
Aromatic compounds								
Toluene	53	84	2.0	1.0	A	0.5–62.5 mg l^{-1}	1:2	‡
Azobenzene	2.5	5.0	0.023	0.009	B	0.023–3 mg l^{-1}	1:2	
1,3,5-trimethylbenzene (mesitylene)	40	~50	2.0	0.4	B	0.125–16 mg l^{-1}	1:2	‡
Halogenated aromatics								
Perchlorocyclopentadient†	0.19	0.21	0.009		A	0.6–75 μg l^{-1}	1:2	‡
2-Chlorophenol	2.0	6.3	0.50	0.30	B and C	0.063–8 mg l^{-1}	1:2	‡
4-Chlorophenol	3.7	8.6	0.63		B	0.08–5 mg l^{-1}	1:2	‡
2-Bromophenol	4.8	13	0.32	0.22	C	0.08–10 mg l^{-1}	1:2	
2-Bromoaniline	2.9	9.0	0.080		B	0.08–20 mg l^{-1}	1:2	
2-Chloroaniline	1.4	6.0	0.032		A, B and C	0.001–3.16 mg l^{-1}	1:$\sqrt{10}$	

2-Bromoaniline	2.9	9.0	0.080	B	0.08–20 mg l^{-1}	1:2				
2-Chloroaniline	1.4	6.0	0.032	A, B and C	0.001–3.16 mg l^{-1}	1:$\sqrt{10}$	**			
3-Chloroaniline	0.40	1.9	0.013	B and C	6.3–800 µg l^{-1}	1:$\sqrt{10}$				
4-Chloroaniline	0.10	13	0.010	B and C	0.1–316 µg l^{-1}	1:$\sqrt{10}$	**			
3,4-Dichloroaniline	0.14	6.0	0.012	B	6–1536 µg l^{-1}	1:2				
2,4-Dichlorophenol	2.8	3.9	0.32	B	0.08–10 mg l^{-1}	1:2				
1,2-Dichlorobenzene	1.0	1.7	0.63	A, B and C	0.08–20 mg l^{-1}	1:2				
1,3-Dichlorobenzene	5.5	7.0	0.80	B	0.1–12.5 mg l^{-1}	1:2				
1,4-Dichlorobenzene	1.5	3.2	0.50	C	0.03–4 mg l^{-1}	1:2				
1-Chloro-2-nitrobenzene	5.0	12	4.0	A, B and C	0.125–16 mg l^{-1}	1:2				
1-Chloro-4-nitrobenzene	3.3	15	0.32	B	0.08–20 mg l^{-1}	1:2				
2-Chlorotoluene	9.0	20	0.27	B and C	0.07–17.5 mg l^{-1}	1:2				
4-Chloro-2-nitrotoluene	7.7	12	0.32	A	0.08–10 mg l^{-1}	1:2				
4-Chloro-3-nitrotoluene	5.5	9.7	0.50	B	0.063–8.0 mg l^{-1}	1:2				
2-Chloro-6-nitrotoluene	2.0	4.0	0.63	A, B and C	0.04–5.0 mg l^{-1}	1:2				
4-Chloro-3-methylphenol (4-chloro-*m*-cresol)	1.9	4.4	1.3	B	0.04–5.0 mg l^{-1}	1:2				
1,2,3-Trichlorobenzene	1.4	>2.0	0.63	C	0.02–2.5 mg l^{-1}	1:2				
Heterocyclic hydrocarbons										
Quinoline	51	76	0.80	B	0.4–50 mg l^{-1}	1:2				

*Where no details are given, test was conducted in an open vessel.
†Nominal concentration was calculated on the basis of the result of chemical analysis.
‡Test in closed vessel.
A—parent animal mortality, B—reproduction rate, C—appearance of first offspring.

The 24 h EC_0 and the 24 h EC_{50} (referred to the nominal value) for the acute *Daphnia* test.

The NOEC as referred to the nominal value and, in addition, the minimum value of the test concentration range, the dilution ratio, the most sensitive parameter and the type of vessel used in the 21 d *Daphnia* reproduction test.

From data on the NOEC and the dilution ratios, it was possible to identify the lowest concentration tested where an effect of the substance could be observed.

Table 2 lists the substances according to their harmful effects (as referred to the nominal value) beginning with the most toxic. The minimum value was also given. In the case of 3 of the 73 toxic substances, the NOEC values in the concentration range comprising 3 logs were between 0.000001 and 0.001 mg l^{-1}, i.e. for ethyl parathion, bis(tri-*n*-butyltin)oxide and for the active ion Cd(II). The NOEC values for 13 of the substances tested were in the concentration range comprising 2 logs, i.e. 0.001–<0.1 mg l^{-1}: potassium dichromate and nickel acetate—as referred to Cr(VI) or Ni(II)—aniline, 2- and 3- and 4-chloroaniline, 3,4-dichloroaniline, 2,4-dimethylaniline, 2-bromoaniline, azobenzene, 2,4-dinitrotoluene, niclosamide and perchlorocyclopentadiene.

In the case of 23 substances, an NOEC was determined in the concentration range 0.1–<1 mg l^{-1}. Of the 73 tested substances, the NOEC for more than half was below 1 mg l^{-1}, the first observed effect under 2 mg l^{-1}.

When compared in terms of position isomerism, 4-chloroaniline was found to have an NOEC 3 times lower than 2-chloroaniline, 3,4-dimethylaniline an NOEC 10 times lower than 2,3-dimethylaniline and *p*-chlorobenzene an NOEC 12 times lower than in the *o*-position.

Table 2 reveals a higher toxicity of phthalates with increasing alkyl chain length. In comparison with phthalic acid diethyl ester, the NOEC of phthalic acid diallyl ester was 4 times lower; it was 13 times lower in the case of phthalic acid dibutyl ester.

Table 3 shows the nominal concentrations obtained for the 24 h EC_{50} and the 21 d NOEC by substance groups. The nominal concentrations had to be given as no results of chemical analysis were available for the 24 h EC_{50}. For each tested substance, the statistically confirmed 24 h EC_{50} and NOEC values were related to each other whereby a substance concentration of NOEC = 1 was used. This table shows that out of the 73 substances tested, the NOEC values for 3 substances were more than 3 logs, for 13 substances more than 2 logs, for 10 substances 50–90 times, for 24 substances 10–40 times and for 14 substances 50–9 times lower than the values for the 24 h EC_{50} of the corresponding substance. According to these findings, the ratio NOEC to 24 h EC_{50} varied between 1:10^3 and 1:2 and was dependent on

Table 2. No observed effect concentration (NOEC) of water pollutants for *Daphnia magna* in the 21 d reproduction test

Pollutant dissolved in synthetic fresh water	Nominal value ($mg\,l^{-1}$)	Minimum value ($mg\,l^{-1}$)
Ethyl parathion	0.000002	
Bis(tri-*n*-butyltin)oxide	0.00016	
Cadmium chloride ($CdCl_2$)	0.0006*	
Perchlorocyclopentadiene	0.009†	
Aniline	0.010	(0.004)
4-Chloroaniline	0.01	
3,4-Dichloroaniline	0.012	
3-Chloroaniline	0.013	
3,4-Dimethylaniline	0.016	(0.01)
Potassium dichromate ($K_2Cr_2O_7$)	0.018*	
Niclosamide	0.020†	
Azobenzene	0.023	(0.009)
2-Choloraniline	0.032	
2,4-Dinitrotoluene (10% water)	0.040	(0.020)
2-Bromoaniline	0.080	
Nickel acetate ($Ni(CH_3COO)_2 \cdot 4H_2O$)	0.090*	
2,4,6-Trimethylphenol	0.10	
3,5-Dimethoxyphenol	0.10	(0.04)
o-Tolidine	0.16	
2,3-Dimethylaniline	0.16	(0.10)
Ethylenediamine	0.16	
2,6-Dinitrotoluene (10% water)	0.16	(0.06)
2-Amino-4-methylphenol (2-amino-*p*-cresol)	0.25	
2-Chlorotoluene	0.27	(0.14)
1-Chloro-4-nitrobenzene	0.32	(0.19)
4-Chloro-2-nitrotoluene	0.32	
2,4-Dichlorophenol	0.32	(0.21)
2-Bromophenol	0.32	(0.22)
4-Chloro-3-nitrotoluene*	0.50	(0.30)
2-Chlorophenol	0.50	(0.30)
1,4-Dichlorobenzene	0.50	(0.30)
Salicylaldehyde	0.63	(0.38)
4-Chlorophenol	0.63	
2-Chloro-6-nitrotoluene	0.63	
1,2-Dichlorobenzene	0.63	
1,2,3-Trichlorobenzene	0.63	(0.03)
Chromium chloride ($CrCl_3 \cdot 6H_2O$)	0.70*	
1,3-Dichlorobenzene	0.80	(0.50)
Quinoline	0.80	
Phthalic acid dibutyl ester	1.0	(0.50)
p-Cresol	1.0	
4,6-Dinitro-*o*-cresol	1.3	
4-Nitrophenol	1.3	
4-Chloro-3-methylphenol	1.3	
Chloramine T	1.3	
Phosphoric acid tributyl ester	1.3	
1-Octanol	1.6	(1.0)
Toluene	2.0	(1.0)
2,4-Dinitrophenol	2.0	
1,3,5-Trimethylbenzene	2.0	(0.40)
2-Nitro-*p*-cresol	2.3	
Isooctanol	2.3	(1.6)
Phthalic acid diallyl ester	3.2	
Monobromoacetic acid	3.2	(1.6)
4-Nitroanisole	3.2	
1-Chloro-2-nitrobenzene	4.0	(3.0)
Chloroacetaldehyde (50% solution)	5.0	
2,4,6-Trinitrophenol (picric acid)	5.0	
Propionic acid ethyl ester	6.3	(1.3)
Propionic acid methyl ester	6.3	(3.2)
Ethyl acetate	12	(2.4)
Chloroform	13	(6.3)
Phthalic acid diethyl ester	13	(3.8)
Nitrobenzene	13	(2.6)
2-Nitroanisole	13	
Sodium fluoride (NaF)	14*	
Triethanolamine	16	
2,3-Dibromo-1-propanol	16	(9.6)
1,3-Dichloro-2-propanol	16	(10.4)
Isobutanol	20	(4.0)
Monochloroacetic acid	32	
Methyl isobutyl ketone	78	(7.8–39)
Sodium bromide (NaBr)	91*	

*Details on concentrations in the test results of inorganic substances are always related to the active ion (with the exception of $K_2Cr_2O_7$ which is related to Cr^{4+}).

†The nominal concentration was calculated on the basis of the result of the chemical analysis of the stock solution.

Table 3. Calculated pollutant concentrations for the 24 h EC_{50} and the 21 d NOEC. Test organism: *Daphnia magna*

Substances in *Daphnia* test	Acute test 24 h-EC_{50} (mg l^{-1})	Reproduction test 21 d-NOEC: Nominal value (mg l^{-1})	EC_{50}/NOEC
Inorganic compounds			
Cadmium chloride: Cd^{2+}	1.9	0.0006	
$CdCl_2$	3.0	0.001	3000
Nickel acetate: Ni^{2+}	21	0.09	
$Ni(CH_3COO)_2 \cdot 4H_2O$	87	0.40	218
Sodium bromide: Br^-	7219	91	
NaBr	9313	117	80
Chromium chloride: Cr^{3+}	22	0.70	
$CrCl_3 \cdot 6H_2O$	111	3.4	33
Sodium fluoride: F^-	352	14	
NaF	777	32	24
Potassium dichromate: Cr^{6+}	0.35	0.018	
$K_2Cr_2O_7$	0.93	0.050	19
Anilines			
4-Chloroaniline	13.0	0.01	1300
3,4-dichloroaniline	6.0	0.012	500
2-Chloroaniline	6.0	0.032	188
3,4-Dimethylaniline	2.9	0.016	181
3-Chloraniline	1.9	0.013	146
2-Bromoaniline	9.0	0.08	113
Aniline	0.9	0.01	90
2,3-Dimethylaniline	10.0	0.16	62
Organophosphorus compounds			
Ethyl parathion	0.002	0.000002	1000
Phosphoric acid tributyl ester *	35	1.3	27
Aromatic nitro compounds			
2,4-Dinitrotoluene (10% water)	38	0.04	950
2,6-Dinitrotoluene (10% water)	20	0.16	125
2-Nitro-*p*-cresol	52	2.3	23
2,4,6-Trinitrophenol (picric acid)	85	5.0	17
4-Nitrophenol	8.0	1.3	6
Nitrobenzene	60	13	5
2-Nitroanisole	65	13	5
4-Nitroanisole	15	3.2	5
2,4-Dinitrophenol	7.0	2.0	3.5
4,6-Dinitro-*o*-cresol	2.3	1.3	2
Aromatic compounds			
Azobenzene	5.0	0.023	217
Toluene	84	2.0	42
2,3,5-Trimethylbenzene(mesitylene)	~50	2.0	25
Esters			
Ethyl acetate	2306	12	192
Propionic acid methyl ester	516	6.3	82
Propionic acid ethyl ester	286	6.3	45
Phthalic acid dibutyl ester	17	1.0	17
Phthalic acid diallyl ester	26	3.2	8
Phthalic acid diethyl ester	86	13	7
Organometallic compounds			
bis(tri-*n*-butyltin)oxide	0.030	0.00016	188
Phenols			
3,5-Dimethoxyphenol	14.0	0.1	140
2,4,6-Trimethylphenol	3.4	0.1	34
2-Amino-4-methylphenol	3.3	0.25	13
p-Cresol	4.9	1.0	5
Amines			
Triethanolamine	2038	16	127
Ethylenediamine	14	0.16	88
o-tolidine	3.2	0.16	20
Niclosamide*	0.16	0.02	8
Chloramine T	4.8	1.3	4
Heterocyclic hydrocarbons			
Quinoline	76	0.8	95
Halogenated aromatics			
2-Chlorotoluene	20	0.27	74
1-Chloro-4-nitrobenzene	15	0.32	47
2-Bromophenol	13	0.32	41
4-Chloro-2-nitrotoluene	12	0.32	38
Perchlorocyclopentadiene*	0.21	0.009	23

continued overleaf

Table 3—*continued*

Substances in *Daphnia* test	Acute test 24 h-EC_{50} ($mg\,l^{-1}$)	Reproduction test 21 d-NOEC: Nominal value ($mg\,l^{-1}$)	Reproduction test 21 d-NOEC: EC_{20}/NOEC
4-Chloro-3-nitrotoluene	9.7	0.5	19
4-Chlorophenol	8.6	0.63	14
2-Chlorophenol	6.3	0.5	13
2,4-Dichlorophenol	3.9	0.32	12
1,3-Dichlorobenzene	7.0	0.8	9
1,4-Dichlorobenzene	3.2	0.5	6
2-Chloro-6-nitrotoluene	4.0	0.63	6
4-Chloro-3-methylphenol (4-chloro-*m*-cresol)	4.4	1.3	3.5
1,2-Dichlorobenzene	1.7	0.63	3
1-Chloro-2-nitrobenzene	12.0	4.0	3
1,2,3-Trichlorobenzene	2.0	0.63	3
Alcohols			
Isobutanol	1250	20	63
1,4-Dichloro-2-propanol	983	16	61
Isooctanol	115	2.3	50
2,3-Dibromo-1-propanol	536	16.0	34
1-Octanol	26	1.6	16
Ketones			
Methyl isobutyl ketone	3623	78	46
Monocarboxylic acids			
Monobromoacetic acid	65	3.2	20
Monochloroacetic acid	96	32	3
Halogenated alkanes			
Chloroform	79	13	6
Aldehydes			
Salicylaldehyde	3.1	0.63	5
Chloroacetaldehyde (50% sol.) *	15.0	5.0	3

*The nominal concentration was calculated from the result of the chemical analysis of the stock solution.

Table 4. Preparation of the stock solution for the tested substances

Substances in *Daphnia* test	Solution in dilution water (DW) for reproduction test ($mg\,l^{-1}$)	Observations
Inorganic compounds		
Cadmium chloride	20	
Potassium dichromate	100	DW without tap water
Chromium chloride	220	DW without tap water
Sodium fluoride	4000	Dist. in H_2O, salts added later to DW
Sodium bromide	20,000	
Nickel acetate	400	
Organometallic compounds		
bis(tri-*n*-butyltin)oxide	0.020	Solubilizer: ethanol 40 mg/10 ml
Organophosphorus compounds		
Phosphoric acid tributyl ester	40	Stirred for 1 h
Ethyl parathion	0.002	Solubilizer: ethanol 10 mg/100 ml
Halogenated alkanes		
Chloroform (trichloromethane)	2000	
Esters		
Ethyl acetate	10,000	
Propionic acid methyl ester	16,000	
Propionic acid ethyl ester	2000	
Phthalic acid diethyl ester	400	Stirred for 1 h
Phthalic acid dibutyl ester	63	Stirred for 24 h, filtered off
Phthalic acid diallyl ester	100	Stirred for 24 h
Alcohols		
Isobutanol	10,000	
Isooctanol	300	Stirred for 1 h
1-Octanol	200	
1,3-Dichloro-2-propanol	4000	
2,3-Dibromo-1-propanol	750	
Phenols		
2,4,6-Trimethylphenol	32	Stirred for 24 h
3,5-Dimethoxyphenol	50	Stirred for 24 h
2-Amino-4-methylphenol (2-amino-*p*-cresol)	30	Stirred for 1 h
p-Cresol	50	DW heated to 40°C, stirred for 2 h

continued on facing page

Table 4—*continued*

Substances in *Daphnia* test	Solution in dilution water (DW) for reproduction test ($mg\,l^{-1}$)	Observations
Aromatic compounds		
Toluene	125	Stirred for 1 h
Azobenzene	6	DW heated to 80°C, stirred for 24 h
1,3,5-Trimethylbenzene (mesitylene)	63	Stirred for 24 h
Halogenated aromatics		
Perchlorocyclopentadiene	15	Stirred for 24 h, filtered off
2-Chlorophenol	32	
4-Chlorophenol	10	
2-Bromophenol	40	
2-Bromoaniline	40	DW heated to 40°C. Stirred for 24 h
2-Chloroaniline	20	Stirred for 24 h
3-Chloroaniline	32	
4-Chloroaniline	63	DW heated to 80°C
3,4-Dichloroaniline	10	Stirred for 24 h in dist. H_2O; filtered off, salts added afterwards to DW
2,4-Dichlorophenol	50	Stirred for 24 h
1,2-Dichlorobenzene	50	
1,3-Dichlorobenzene	50	Stirred for 24 h
1,4-Dichlorobenzene	40	DW heated to 60°C, stirred for 24 h
1-Chloro-2-nitrobenzene	50	Stirred for 24 h
1-Chloro-4-nitrobenzene	50	Stirred for 24 h
2-Chlorotoluene	80	Stirred for 24 h
4-Chloro-2-nitrotoluene	10	Stirred for 24 h
4-Chloro-3-nitrotoluene	15	Stirred for 24 h
2-Chloro-6-nitrotoluene	10	DW heated to 40°C, stirred for 24 h
4-Chloro-3-methylphenol (4-chloro-*m*-cresol)	10	Stirred for 24 h
1,2,3-Trichlorobenzene	2.5	DW heated to 80°C, stirred for 24 h
Heterocyclic hydrocarbons		
Quinoline	100	DW heated to 80°C
Aldehydes		
Chloroacetaldehyde	20	
Salicylaldehyde	100	
Amines		
Ethylenediamine	20	
Triethanolamine	2000	DW heated to 30°C
Aniline	32	
2,3-Dimethylaniline	50	
3,4-Dimethylaniline	200	Stirred for 24 h
o-Tolidine	10	Stirred for 24 h, filtered off
Chloramine T	40	
Niclosamide	2	Stirred for 24 h, filtered off
Monocarboxylic acids		
Monochloroacetic acid	400	
Monobromoacetic acid	100	
Ketones		
Methyl isobutyl ketone	5000	
Aromatic nitro compounds		
Nitrobenzene	400	Stirred for a few hours
2-Nitroanisole	200	Stirred for 1 h
4-Nitroanisole	50	Stirred for 24 h
4-Nitrophenol	50	
2,4-Dinitrophenol	15	
2,4,6-Trinitrophenol (picric acid)	160	Stirred for 1 h
2,6-Dinitrotoluene (10% water)	20	Stirred for 24 h
2,4-Dinitrotoluene (10% water)	20	DW heated to 80°C, stirred for 24 h
2-Nitro-*p*-cresol	200	Stirred for 24 h
4,6-Dinitro-*o*-cresol	13	Stirred for 24 h

the substance. In the first group, cadmium chloride achieved the highest ratios followed by 4-chloroaniline and ethyl parathion. The second group contained an organic tin compound and nickel acetate and azobenzene, five aniline compounds and two dinitrotoluenes.

In the 21 d reproduction test, the anilines showed a higher than average potentially harmful effect than in the acute *Daphnia* test and thus distinguished themselves very clearly from the other substance groups.

If one compared the ratio NOEC/24 h EC_{50}, then this meant that in terms of position isomerism, 4-chloroaniline had a 7 times more harmful effect than 2-chloroaniline and a 9 times more harmful effect than 3-chloroaniline and that 3,4-dimethyl-

aniline had a 3 times more harmful effect than 2,3-dimethylaniline.

In the case of the aromatic nitrocompounds, 2,4-dinitrotoluene had an approx. 8 times more harmful effect than 2,6-dinitrotoluene. In the group of halogenated aromatics, chloronitrobenzene in the *p*-position had a 15 times more harmful effect than in the *o*-position and *m*-dichlorobenzene a 3 times more harmful effect and *p*-dichlorobenzene a 2 times more harmful effect than in the *o*-position.

EVALUATION OF THE TEST METHOD

In determining the reproduction rate, the survival rate of the parent animals over a period of 21 days and the time of the appearance of the first offspring, this test has three parameters which are easily quantifiable and which can be related to the control preparations. In order to determine the NOEC in the 73 substances tested, the reproduction rate—being the most sensitive parameter—was used in 58 cases, on 29 of these together with one or both other parameters. By contrast, in 7 cases the parent animal mortality and the appearance of the first offspring, respectively, proved to be the most sensitive parameters.

The validity criteria were met as follows. Altogether, 64 control solutions in beakers and 29 control solutions in closed glass bottles with ground-glass stoppers were prepared. The reproduction rate per parent animal after 21 days, in the case of the test preparation in the beakers, was 88.8 offspring (SD = 13.1; coefficient of variation = 14.8%), in the bottles it was 68.0 offspring (SD = 10.6%; coefficient of variation = 15.6%). The "parent animal mortality" after 21 days was 7.1% in the case of the test preparation in beakers and 9.1% in bottles. The "first offspring" appeared in both types of vessel on the 7th, and only in a few cases, on the 8th test day at the very latest. Both met the quality criteria.

The oxygen content and pH value of the test and control media can influence the test organisms and thus, they were measured on each transfer day. Evaluation of the measured data revealed the following. On no occasion was the pH value—based on 8.0 ± 0.2—lower than 7.0 in any of the control or test preparations either in beakers or in bottles at the end of the test period, i.e. after 48/72 h; it always remained in the neutral to subalkaline range. Based on the oxygen saturation of the test and control media, an average minimum oxygen saturation value of 69% was measured at the end of the test period in the beakers, and a value of 58% in the bottles. A negative influence on the test organisms could be ruled out even at these extreme values.

When carrying out the 21 d *Daphnia* reproduction test, the chemical quantification of substance concentrations at selected dilution steps proved necessary in order to ensure that the results could be interpreted reliably.

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EFFECTS OF FLUORIDE ON GROWTH, REPRODUCTION AND SURVIVAL IN *DAPHNIA MAGNA*

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Abstract—1. The effects of waterborne fluoride (NaF) on growth, reproduction and survival in *Daphnia magna* were studied from subnormal to toxic concentrations in hard reconstituted water.

2. The 24- and 48-hr TC_{50}s for immobilization were 205 and 98 mg F (fluoride) l^{-1}. Median survival times for fed and unfed *Daphnia* were reduced at concentrations of F above 8.9 and 10 mg Fl^{-1}, respectively.

3. Growth, determined as body length after 7 and 21 days, was partially inhibited at all concentrations above 3.7 mg Fl^{-1}.

4. Parthenogenetic reproduction was stimulated by all concentrations (dilution factor 0.5) between 0.45 × 10^{-3} mg Fl^{-1} and 3.7 mg Fl^{-1} and inhibited by all concentrations above 3.7 mg Fl^{-1}, compared to the control with no waterborne fluoride. The highest concentration with a reproduction (number of live progeny less female) equivalent to the control after 21 days was 4.4 mg Fl^{-1}. However, a progressive decline in reproduction between 14 and 21 days indicates a slight long-term inhibition above 0.58 mg Fl^{-1}.

5. The "safe" concentration equivalent to the geometric mean of NOEC or MATC for *D. magna* in hard water is 4.4 mg Fl^{-1}, derived as ZEP, the Zero Equivalent Point, for reproduction after 21 days.

INTRODUCTION

Fluorine is the most electronegative of all elements and does not normally occur as a free element in nature. The concentration of fluorine in the earth crust is about 0.6–1 g kg^{-1}. The most abundant minerals are apatite $Ca_5(OH, F)(PO_4)_3$, where OH^- and F^- can substitute each other, fluorite CaF_2, and tryolite Na_3AlF_6. The concentration of fluorine in sea water is 1.2–1.4 mg l^{-1}, regardless of oceanic location (Dobbs, 1974), even if somewhat higher concentrations have been found in some deep areas of the Atlantic (Greenalgh and Riley, 1963); Brewer *et al.*, 1970). Most surface waters contain less than 0.2 mg l^{-1} dissolved fluoride and the majority contain less than 0.1 mg l^{-1}. Total concentrations can be considerably higher as much of the fluoride is bound to small suspended particles (Borg, 1976). According to Dobbs (1974) most of the dissolved fluoride in inland waters and rivers come from atmospheric precipitation, which in turn could derive it from the sea, volcanic activity or industrial pollution. Emissions of fluorine occur in the production of aluminium, fertilizers, steel, glass, brickstones, ceramics, fluorine compounds (especially HF), fluorinated hydrocarbons and polymers, cement, glass fibre, wood preservatives and during combustion of fossil fuels and garbage (Borg, 1976).

Toxic effects of fluorine compounds have been described in plants, fishes, insects, amphibians, fish and mammals (reviews by Dobbs, 1974; NAS-NRC, 1974; Groth, 1975; Borg, 1976). Several fluoride compounds are potent enzyme inhibitors. Fluoride is mainly deposited in bone. In aquatic animals elevated residues will result from the uptake of fluorine from water (Hemens and Warwick, 1972; Hemens *et al.*, 1975; Moore, 1971; Neuhold and Sigler, 1960; Sigler and Neuhold, 1972; Wright and Davidson, 1975) and from food (Grave, 1981; Taves *et al.*, 1982). In marine fish the skeleton and the skin had the highest levels and in crustaceans the exoskeleton (Wright and Davidson, 1975).

In fish, the acute toxicity of fluoride is influenced by abiotic factors like hardness (Neuhold and Sigler, 1960; Herbert and Shurben, 1964), chloride concentration and temperature (Angelovic *et al.*, 1961) and by biotic factors like developmental state, size and species (Neuhold and Sigler, 1960). Signs of acute fluoride intoxication were anorexia, increased secretion of mucus and loss of equilibrium accompanied by tetany and death. In the blood, the total serum protein and calcium decreased, while magnesium and alkaline phosphatase increased. In rainbow trout (*Salmo gairdneri*) the epithelium of the gill filaments and the skin were affected and the ultimobranchial gland, which is involved in calcium regulation, was enlarged after exposure to 25 mg Fl^{-1} (Sigler and Neuhold, 1972). In the leopard frog concentrations above 50 mg Fl^{-1} (as NaF) affected mobility and posture. Higher concentrations produced weakening of righting reflexes, local haemorrhages in stomach and intestine, enlargement of heart and gall bladder, and microcoronation of blood vessels, liver and urine. The heart and respiratory rates were not affected below 200 mg Fl^{-1}. Nevertheless, at concentrations between 5 and 50 mg Fl^{-1} the total counts of red and white blood cells showed a progressive decline, the latter because of granulopenia (Hopkins *et al.*, 1964). Metamorphosis and thyroid activity in tadpoles of *Rana temporaria* were inhibited by 1, 2 and 10 mg Fl^{-1} (Kuusisto and Telkä, 1961), and survival of "Jack Dempsey" (*Cichlasoma biocellatum*) larvae was progressively reduced at 2.5 mg Fl^{-1} and higher

concentrations (Hasselrot, 1956). Thus, sublethal effects in adult amphibians and fishes have been seen at 5 and 25 mg F^{-1} (as NaF), and sensitive developmental stages have been affected at only a few milligrams of fluoride per litre.

The sensitivity of freshwater crustaceans to fluorine compounds has, to my knowledge, only been studied in *Cladocera*. In soft reconstituted water, the 48-hr LC_{50} for cryolite (Na_3AlF_6) was 5 mg l^{-1} in *Daphnia pulex*, and 10 mg l^{-1} in *Simocephalus serrulatus* (Sanders and Cope, 1966). In tests with NaF in hard water, Reingmann and Kuhn (1959) found a 48-hr LC_{100} of 270 mg NaF l^{-1}, and Le Blanc (1980) found 24- and 48-hr LC_{50}s and a "no discernible effect concentration" of 680, 340 and 110 mg NaF l^{-1}, respectively, in *Daphnia magna*. Previous studies in marine crustaceans (Hemens and Warwick, 1972; Wright and Davidson, 1975) have not shown acute lethality below 30 mg l^{-1} and no long-term effects were found by Hemens *et al.* (1975) at 5.5 mg l^{-1} and by Connel and Airey (1982) at 4.15 mg l^{-1}. In the blue crab (*Callinectes sapidus*), concentrations of 20 mg l^{-1} (as NaF) and above, added to natural sea water, reduced growth, and concentrations above 20 mg l^{-1} resulted in accumulation in muscle tissue. Significant accumulation occurred in exoskeleton, gill, hepatopancreas and muscle tissues at 8, 32 and 128 mg l^{-1}, while at 2 mg l^{-1}, the tissue fluoride content remained basically unchanged (Moore, 1971).

In the present study the chronic effects of nearly acute toxic concentrations down to concentrations below the natural freshwater concentrations have been examined in *D. magna*. All exposures were made in a hard reconstituted water (ISO, 1982), which made it possible also to study the effects of fluorine deficiency and to investigate the complete dose-response pattern for effects on growth, reproduction and survival.

MATERIALS AND METHODS

Animals

Daphnia magna has been cultured at our laboratory for several years. We used four aquaria with 25 l reconstituted hard water (Table 1) supplemented with 1 l natural tap water. Each aquarium contained *D. magna* of 0–1, 1–2, 2–3 and 3–4 weeks of age respectively. All cultures were aerated through a glass tube, kept at room temperature and illuminated by two 40 W ordinary tubes 50 cm above the surface for 12 hr a day. All cultures were fed a 2:1 mixture of Dorowall dry larval fish food (Roswall-Produkte AG, Zürich, Federal Republic of Germany) and Findus dry baby gruel (Produits Findus S.A., Vevey, Switzerland) once daily by an automatic feeder (Readomatic®, Gelsdin, Federal Republic of Germany). The daily ration of dry food was 480 mg to each aquarium. A new culture was started each week with neonates from the oldest culture. Approximately 5 l of the old medium was retained and supplemented with

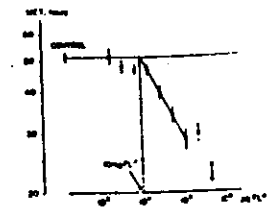

Fig. 1. Median Effective Times, METs, for immobilisation of unfed *Daphnia magna* in various concentrations of fluoride

25 l reconstituted hard water and 1 l natural sea water; these means the cultures were maintained on a 4-week cy... The neonates used in the experiments were taken from ... culture with females, 3–4 weeks old.

Acute toxicity

Two acute toxicity tests were made by exposing 1... neonates (6–24 hr old) in 200 ml test solution kept ... ordinary glass beakers covered with watch glasses. ... solutions were made from sodium fluoride (NaF, *pro ...* ... Merck, M = 42.0 g mol^{-1}) and hard reconstitu... water (Table 1). The dilution factor was 0.5. The ... perature (20 C) was regulated by a thermostat and ... mination was the same as for the cultures. The first ... generated 24- and 48-hr EC_{50}s and the second test a ... curve for unfed neonates (Fig. 1).

Chronic toxicity

In the chronic test, 25 neonates (6–24 hr) with a ... length (see below for determination) of 0.90 mm (SD ... N = 7) were exposed to each of 20 concentrations of ... fluoride (NaF as above) in hard reconstituted water (... 1). The test solutions (1 l) were kept in ordinary 1.5 l ... beakers immersed in a water bath (20 C) and illumin... above. The food solution, which was added at 0.3 ... prior to the light was turned off, consisted of the soluble ... suspended portion of a homogenized mixture of 50 g ... SM 80 tropical fish food flakes (Tetra Werke, ... Republic of Germany) and 25 g Findus baby gru... above) in 1 l deionized water. This gave an initial ... tration of approximately 30 mg dry food l^{-1}. ... solutions were completely renewed Mondays, Wedn... and Fridays throughout the test. The number of ... dissolved oxygen and pH were also recorded at ... occasions.

After seven days 10 daphnids from each ... were transferred to separate petri dishes of glass (6.4 ... containing 50 ml test solution. The remaining daphn... used for determination of body length as describe... The test solutions were renewed as above and the ... of live progeny was also recorded. Temperature ... trolled by a thermostat and the dishes were illumin... 12 hr/day. The same food solution as above was ... each dish on renewal at 30 µl/dish.

Table 1. Chemical composition of hard reconstituted water used to assess acute and chronic toxicity with *Daphnia magna*

Ingredient	$CaCl_2 \cdot 2H_2O$	$MgSO_4 \cdot 7H_2O$	$NaHCO_3$	KCl
Concentration	294	123	64.8	5.8

Alkalinity: 2.4 mEq l^{-1} (= 120 mg/l as $CaCO_3$)
Hardness: 270 mg l^{-1} as $CaCO_3$

The test was terminated after 21 days and the body length was determined for all surviving adults. Body length (upper part of the eye to base of caudal spine) was determined from a photo taken through a dissection microscope.

Statistical treatment

Median effective times for immobilization (METs) or survival (MSTs), median effective concentrations for immobilization (EC_{50}), their 95% confidence limits (CLs) and slopes (S) were determined by probit analysis on a microcomputer according to Davies (1971). Nominal concentrations of sodium fluoride (NaF, M = 42.0 g mol^{-1}) were used and calculated back to fluorine (F, M = 19.0 g mol^{-1}). Values for body length and reproduction per female are given as mean values (x) ± standard error (SE) and number of daphnids (N). The values for reproduction were calculated from the number of live progeny and the number of live females at the previous observation.

RESULTS

On addition of sodium fluoride to the dilution water, a precipitate, presumably of CaF_2, developed slowly at higher concentrations. The presence of precipitation, and measured intervals for dissolved oxygen, pH and temperature in the acute and chronic tests are shown in Table 2. Addition of NaF in the highest concentrations produced a slight increase of pH, which was not compensated for by addition of acid.

Acute toxicity

The first gave a 24-hr EC_{50} of 454 mg NaF l^{-1} (CL 384–537, S 2.51) and a 48-hr EC_{50} of 216 mg NaF l^{-1} (CL 174–267, S 2.49). There was no effect in the control. Our values are slightly lower than those reported by LeBlanc (1980) of 680 and 340 mg NaF l^{-1}, respectively. Our 24- and 48-hr EC_{50}s correspond to 205 and 98 mg F (fluoride) l^{-1}.

The results from the second test are presented in Fig. 1. The intersection between the control MET (horizontal line) and the dose-response curve for the higher concentrations occurs at a nominal concentration of 10 mg $F l^{-1}$. It should be observed that 3.7 and 7.4 mg $F l^{-1}$ gave shorter METs than the control, even if the differences are small and there is an overlap in CL for 3.7 mg $F l^{-1}$ and the control. There is no apparent threshold, and the NOEC (No Observed Effect Concentration) for immobility in unfed neonates is between 3.7 and 7.4 mg $F l^{-1}$. The interaction value of 10 mg $F l^{-1}$ is the Zero Equivalent Point (ZEP) for the same response variable.

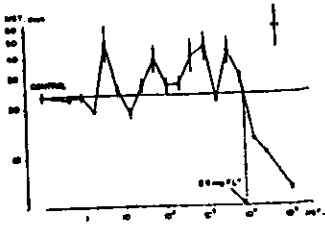

Fig. 2. Median Survival Times, MSTs, for fed *Daphnia magna* in various concentrations of fluoride.

Chronic toxicity

The same reconstituted water was used in both the acute and the chronic test. In the chronic test the addition of food made small amounts of other minerals available. It also produced lower values for dissolved oxygen and pH as seen in Table 2. The lowest values occurred during the first week, when beakers were used. During the second and third week, when petri dishes were used, the decrease in dissolved oxygen and pH between renewals was less.

Effects on survival

Survival in higher concentrations of sodium fluoride was higher in the chronic (Fig. 2) compared to the acute test (Fig. 1). The presence of food in the chronic test made it possible for the daphnids to grow. During growth the exoskeleton is shed in each moult, and moulting is an efficient way to eliminate fluoride, which in crustaceans is incorporated into the exoskeleton, in particular (Moore, 1971; Wright and Davidson, 1975).

When the *Daphnia* reproduction test is made in reconstituted water instead of in natural water, the conditions for reproduction and survival are less favourable, and mortality data for the control as well as lower concentrations can be used to determine MST. However, since less than 50% mortality occurred in the control and lower concentrations of F, all MSTs higher than 21 days in Fig. 2 are extrapolated values and consequently less reliable. The NOEC is between 7.4 and 14.8 mg $F l^{-1}$ and ZEP is 8.9 mg $F l^{-1}$ for survival in fed daphnids. Considering

Table 2. Dissolved oxygen, pH and temperature in acute and chronic tests with sodium fluoride

Type of test	Parameter	Range in all concentrations
Acute	Dissolved oxygen	90–100% of air saturation
	pH	7.9–8.8
	Temperature	20.0–20.8 C
	White precipitate	Observed in concentrations ≥ 30 mg $F l^{-1}$
Chronic	Dissolved oxygen	20–100% of air saturation
	pH	8.0–8.8
	Temperature	19.5–21.5°C
	White precipitate	Observed in concentrations ≥ 7.4 mg $F l^{-1}$

tration would probably define the optimum concentration more precisely, and possibly shift the entire dose response curve slightly towards lower concentrations.

Toxicity and safe concentration philosophy

The lethal effect of a toxicant appears when stress-induced compensatory mechanisms have been exhausted and a sufficient dose has affected the most sensitive function of vital importance for the organism. In this study, the lethal effect of fluoride was delayed upon addition of food, presumably because of the increased elimination of fluoride through the thrown exoskeletons in continuously growing *Daphnia*. However, survival in relation to respective controls in fed and unfed *Daphnia* was affected at similar concentrations (8.9 and 10 mg $F l^{-1}$).

The sublethal effects of a toxicant are more difficult to interpret because both primary toxic effects and secondary compensatory effects are often expressed in the same response variable, but in opposite directions. Thus, the increase in a measured variable, like growth, reproduction or even survival, does not necessarily imply that the organism is performing better. It only implies that it is performing differently from the control. In most toxicity tests, it is assumed that the control is provided with optimal conditions, but apparently this is not the case. In the extensive chronic study on *D. magna*, Biesinger and Christensen (1972) wrote that "reproduction was usually stimulated by small metal additions", and the inhibition of reproduction (16 and 50%) was not compared to the control but to the optimum concentration. Since the optimum concentration was not given, the effect might have been related to the hormetic spike or to the optimal concentration, and this could make a considerable difference.

I agree with Stebbing (1981) and Luckey (1975) that hormesis seems to be a frequently occurring phenomenon. From a toxicological point of view, the question is if this stimulation is beneficial or detrimental. Stebbing suggested that hormesis resulted from an effect on the growth control mechanism. But there is no real evidence for Stebbing's more general explanation. Nor is there evidence for previously suggested biochemical or physiological explanations. From an ecological point of view, hormesis might be beneficial to the organism and to the ecosystem, especially if it is a short-term response. One such situation would be when an essential nutrient suddenly appears at high and even toxic concentrations. Then it should be incorporated more efficiently and saved in the biomass for an extended period. Even if it is a non-essential accumulating chemical, the hormetic spike will increase the biomass and reduce toxicity through growth dilution. From a physiological point of view, it might be argued that hormesis is a symptom of stress, which in a longer perspective is detrimental to the organism. The results from this study do not exclude any of these possibilities. What they show is that it is important to distinguish an essentiality from a hormetic spike.

The aim of a toxicity test is to determine the negative effects of a chemical. In chronic tests with aquatic organisms the "safe" concentration is commonly expressed as MATC (Maximum Acceptable Toxicant Concentration, Mount and Stephan 1967) or NOEC (No Observed Effect Concentration, Maki, 1979). Both expressions are the interval between the highest concentration with no significant effect and the lowest concentration with a significant effect. One disadvantage about this definition is that the result is an interval instead of a single value. Further, this interval is not an estimate of the error but instead the results of the preselected test concentrations, the CV of the response variable and the sample size (N).

Luckey (1975) realized the general occurrence of hormesis and proposed that ZEP, the Zero Equivalent Point, ought to be a legally defensible value. In my opinion, it would be a better rationale to use ZEP instead of MATC or NOEC as a substitute for the truly safe concentration. The rationale of using ZEP as a "safe" concentration is "that the highest concentration with a response identical to the control would provide an adequate protection for the organism as regards the measured response variable". In contrast to MATC and NOEC, ZEP is dependent on the averages instead of the error, and because of that no sophisticated statistical analyses is needed.

In the graphic determination of ZEP, the hormetic spike is advantageous because ZEP becomes an interpolated value. For its determination, the experimental design should be directed towards the use of more concentrations, but with fewer replications. This would probably be cost-beneficial because the toxicity tests with MATC values at concentrations greater or smaller than 1 mg l^{-1} would be fewer. In most cases ZEP and the geometric mean of the values delineating MATC and NOEC would be the same. But environmental authorities would probably appreciate single values instead of ranges for single species and a single figure for an entire ecosystem in the chronic water quality criteria seems illogical.

CONCLUSIONS

(1) Low concentrations of fluoride stimulated reproduction in *D. magna*. The minimum requirement of waterborne fluoride in reconstituted water with a hardness of 250 mg l^{-1} as $CaCO_3$ in *D. magna* and artificial diet was 4 µg $F l^{-1}$. In addition 10–70% stimulation called "hormesis", which is a non-specific physiological stimulation at sublethal doses of concentrations.

(2) The Zero Equivalent Point (ZEP), which highest concentration with a response identical control, for survival in unfed and fed *D. magna* 10 and 8.9 mg $F l^{-1}$, for growth after 7 and about 3.7 mg $F l^{-1}$ and for reproduction after 4.4 mg $F l^{-1}$.

(3) In the absence of other toxicants, trations of fluoride above 4.4 mg $F l^{-1}$ in hard would have a significant negative impact growth and reproduction of *D. magna*, and long-term effect on reproduction is expected 0.6 mg $F l^{-1}$. Concentrations between 0 4.4 mg $F l^{-1}$ stimulate reproduction by hor

(4) The "safe" concentration equivalent geometric mean of NOEC or MATC for *D.* hard water is 4.4 mg $F l^{-1}$, derived as ZEP for reproduction after 21 days.

Acknowledgements—This investigation has been supported by the National Swedish Environment Protection Board (contract 5313012-2) and the University of Gothenburg (E 38a 2700 82). The experiments were carried out by Mrs Eva Nilsson. The figures were drawn by Mrs Birgitta Vallander. I am extremely grateful for the assistance offered by the organizations and persons mentioned above.

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the irregularities in the dose response relationships and the absence of threshold in Fig. 1, the NOECs and ZEPs for acute and chronic survival are very similar

Effects on growth

The results in body length after 7 and 21 days are presented in Table 3 and Fig. 3. The NOEC for growth after both 7 and 21 is estimated to be between 3.7 and 7.4 mg $F l^{-1}$. The higher variability in size after 7 compared to 21 days might be due to the smaller 7-day samples. Thus, the complex pattern of both inhibition and stimulation is less reliable. After 21 days the daphnids reached the same size at all subinhibitory concentrations.

Effects on reproduction

The actual number of progeny per female in the control can vary from one test to another. In our experience the number in this study (Table 3) is about the average when this reconstituted water and this food is used. The present results suggest an essentiality of fluoride in *D. magna*. The curves presented in Fig. 4 are rather complex with several peaks of stimulation. Although the SE is large for most concentrations, the pattern of the curves suggests that one should look for a biological explanation and not only regard the peaks as random variations. Part of the explanation must be that fluoride is accumulated slowly, making the internal distribution and dose into a function of both concentration and time. True equilibria are not reached in the lower sublethal concentrations. A slow accumulation, in combination with essentiality as well as toxicity, can explain the peak in reproduction at just subinhibitory concentrations around 1 mg $F l^{-1}$ after 14 days. The stimulating effect at 0.9, 1.8 and 3.6 μg $F l^{-1}$ after 14 days and at 0.5, 0.9, 1.8, 3.6 and 7 μg $F l^{-1}$ after 21 days suggests an essentiality in the absence of toxicity. The minimum requirement is about 4 μg $F l^{-1}$. The NOEC for effect on parthenogenetic reproduction is between 3.7 and 7.4 mg $F l^{-1}$ and the ZEP is 4.4 mg $F l^{-1}$. The fluctuations in reproduction in the broad three orders of magnitude interval between 5 μg $F l^{-1}$ and 5 mg $F l^{-1}$ might be a mixture of essentiality, hormesis and inhibition under the influence of concentration of fluoride and period of exposure.

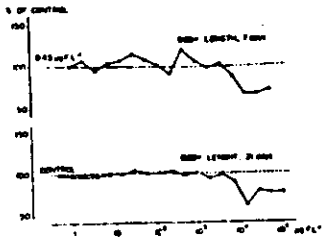

Fig. 3. Effect of fluoride on growth in *Daphnia magna*

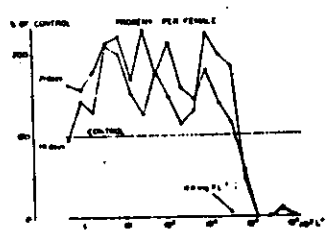

Fig. 4. Effects of fluoride on reproduction in *Daphnia magna*

Table 3. Effect of monolevel fluoride (NaF) on growth, reproduction and survival in *Daphnia magna*

Concentration (μg $F l^{-1}$)	Body length (mm)* 7 days	14 days	Progeny per female* 14 days	21 days	Survival (%)* 7 days	14 days	21 days
0 (control)	Not determined	3.00 ± 0.00 (50)	8.7 ± 2.1 (9)	19.0 ± 3.9 (8)	92	88	28
0.45	2.13 ± 0.07 (4)	3.00 ± 0.00 (40)	8.3 ± 2.9 (9)	20.8 ± 0.8 (6)	88	88	88
0.9	2.14 ± 0.08 (3)	2.90 ± 0.10 (10)	12.4 ± 3.5 (9)	20.7 ± 2.3 (7)	100	88	88
1.8	2.28 ± 0.13 (5)	2.92 ± 0.08 (9)	11.3 ± 2.3 (9)	21.8 ± 0.5 (5)	100	88	88
3.6	2.03 ± 0.03 (3)	2.94 ± 0.06 (7)	10.2 ± 4.1 (9)	20.5 ± 1.5 (8)	100	88	88
7	2.22 ± 0.10 (5)	3.03 ± 0.03 (6)	13.3 ± 3.4 (8)	42.0 ± 7.0 (8)	100	88	88
15	2.20 ± 0.27 (3)	3.00 ± 0.00 (4)	13.2 ± 2.3 (10)	33.2 ± 5.0 (6)	88	88	88
30	2.04 ± 0.00 (2)	3.15 ± 0.07 (4)	11.8 ± 4.3 (7)	43.3 ± 10.4 (6)	88	88	88
58	2.31 ± 0.02 (3)	3.00 ± 0.25 (4)	6.8.3 ± 2.1 (10)	35.0 ± 9.1 (7)	88	88	88
116	2.18 ± 0.09 (2)	3.07 ± 0.08 (6)	12.9 ± 2.1 (8)	40.4 ± 5.9 (7)	88	88	88
232	1.86 ± 0.18 (4)	3.13 ± 0.04 (6)	9.9 ± 2.1 (8)	26.9 ± 8.3 (7)	88	88	88
464	2.38 ± 0.10 (3)	2.90 ± 0.12 (5)	11.3 ± 2.3 (9)	27.4 ± 5.3 (7)	100	88	88
926	2.37 ± 0.13 (3)	3.05 ± 0.07 (5)	10.6 ± 2.9 (10)	34.0 ± 5.4 (7)	88	88	88
1853	2.11 ± 0.07 (4)	2.84 ± 0.04 (5)	13.1 ± 4.0 (9)	26.9 ± 6.7 (6)	88	88	88
3706	2.19 ± 0.17 (4)	2.97 ± 0.07 (8)	13.9 ± 2.7 (10)	20.8 ± 4.3 (9)	88	88	88
7412	1.86 ± 0.16 (9)	2.60 ± 0.06 (7)	4.9 ± 1.5 (10)	11.8 ± 3.2 (7)	92	88	88
14,824	1.42 ± 0.10 (4)	1.86 (1)	0 (4)	0 (1)	88	88	88
29,647	1.43 ± 0.08 (3)	0.36 ± 0.20 (3)	0 (6)	0 (2)	88	88	88
59,294	1.61 ± 0.09 (4)	0.33 ± 0.08 (6)	0.0 ± 0.0 (10)	0.0 ± 0.0 (6)	88	88	88
118,589	Not determined	0.00 ± 0.00 (7)	0 (0)	0.3 ± 0.3 (3)	36	25	11

*$\bar{x}$ ± SE (N).
†Corrected for tolerance below 25 to 30 water fleas after 3 days.

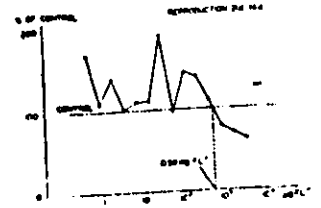

Fig. 5. Effect of fluoride on 21-14 day reproductive ratio in *Daphnia magna*

The importance of concentration and time for the effect of fluoride on reproduction can be seen in Fig. 5. In the concentrations between 0.45 and 463 μg $F l^{-1}$ reproduction between 11 and 21 days was either linear or progressively increased. In the interval between 926 and 3710 μg $F l^{-1}$ an initial stimulation was followed by partial inhibition after about 14 days, and at 14,800 μg $F l^{-1}$ and above inhibition was almost complete. A simplified numerical estimate of an altered reproductive rate during the experiment is the 21-14 day progeny/female ratio, which is shown in Fig. 5. The NOEC based on this ratio is between 463 and 926 μg $F l^{-1}$ and the ZEP is 580 μg $F l^{-1}$. This ratio was the most sensitive estimate of long-term fluoride toxicity in this study.

DISCUSSION

The present study has shown that concentrations of fluoride above 9 mg $F l^{-1}$ resulted in reduced survival, and above 4.4 mg $F l^{-1}$ in reduced growth and reproduction in *D. magna*, when compared to the control in hard reconstituted water without fluoride. This estimate of chronic toxicity at concentrations above 4.4 mg $F l^{-1}$ is consistent with previous findings in mullet, *Mugil cephalus*, the prawn *Penaeus indicus*, the crab *Trichodactyl hilpharatinus* (Hemens *et al.*, 1974a and b) and the estuarine amphipods *Grandidierella lutosa* and *G. lignorum* (Connell and Airey, 1982). However, the long-term decline in *Daphnia* 1982). However, the long-term decline in *Daphnia* reproduction at higher concentrations suggests that concentrations as low as 0.6 mg $F l^{-1}$ could affect long-term reproduction in *D. magna*.

The evaluation of fluoride toxicity in *Daphnia* was complicated by the stimulating effects at sublethal concentrations. This study is unique as regards the broad range of concentrations that has been examined. The reason for making such a study was the previous findings that low levels of toxicants are often stimulating at subinhibitory concentrations. Such stimulation can arise because of essentiality but also because of hormesis. The latter is physiological stimulation by a subinhibitory amount of a chemical or physical agent (Luckey, 1975). It seems to be a more frequent occurrence in aquatic toxicology than in generally expected (Stebbing, 1981).

Essentiality

An essentiality of fluoride in animals has not been conclusively demonstrated so far (NAS-NRC, 1974). However, Messer *et al.* (1972a) found an impaired fertility in mice at 0.1–0.3 ppm F in the diet and a more severe anaemia induced by the stress of pregnancy and growth when the fluoride intake was low (Messer *et al.*, 1972b). Furthermore, Schwartz and Milne (1972) found a favourable growth response in rats on addition of small increments of fluoride (1, 2.5 and 7.5 ppm F) to the diet, and trace quantities of F is beneficial for development of caries-resistant teeth and may also be beneficial in preventing excessive demineralization of bone (osteoporosis) in aged individuals (NAS-NRC, 1974).

The present study suggests an essentiality of fluoride in *D. magna*. The minimum requirement is fulfilled at about 4 μg l^{-1}, which is far below the tonic level of 0.5 to 5 mg $F l^{-1}$. *Daphnia magna* cultured in natural spring water with 19 μg $F l^{-1}$ and fed algae containing 9.9 or 13.4 mg F kg^{-1} dry weight, contained 9.4 mg F kg^{-1} dry weight which is equivalent to 50 mg F kg^{-1} wet weight. In reference to Cl in the spring water, the algae were enriched in F and the *Daphnia* excluded F (Cowgill, 1976). The very low requirement of F in *D. magna* of 4 μg $F l^{-1}$ is provided by most natural surface and ground waters. Thus, in nature the dietary contribution of F might be important but probably not essential in *Daphnia*. On the contrary, ground waters in some regions may contain toxic levels of F (Pal, 1982).

Hormesis

There are very few systematic studies on the effect of chemicals covering such a broad range of concentrations as in this study. Luckey (1975) has suggested four different patterns (α, β, γ and δ) of biological response to various concentrations or doses of a chemical. Thus, there is no single dose–response model applicable to the entire range of concentrations examined in the present study.

The peak in reproduction between 232 and 7412 μg $F l^{-1}$ after 14 days (Fig. 4) is a typical example of hormesis, a stimulation that occurs at concentrations or doses just below those that are inhibitory (Stebbing, 1981). A similar stimulation of estuarine amphipod populations (*Grandidierella lutosa* and *G. lignorum*) with fluoride has been described by Connel and Airey (1982). There are two additional peaks in reproduction after 14 days, one around 3.6 μg $F l^{-1}$ and another, smaller one around 58 μg $F l^{-1}$, and there is no apparent, single optimal interval.

After 21 days, the hormetic spike around 1000 μg $F l^{-1}$ has almost disappeared (Fig. 4), because of the delayed inhibition of reproduction at these concentrations (Fig. 5). The dose–response curve after 21 days is what would be expected for an essential element, with a zone of deficiency below 4 μg $F l^{-1}$, an optimal zone between 4 and 116 μg $F l^{-1}$, a zone of sublethal toxicity between 116 and 3706 μg $F l^{-1}$, with a remnant of the 14 day hormetic spike between 463 and 1853 μg $F l^{-1}$, and a zone of lethal toxicity between 3706 and 14,824 μg $F l^{-1}$. Because of the tendency for long-term and slow accumulation of fluoride in crustaceans (Moore, 1971), a multigeneration exposure with a larger number of animals at each concen-

FLUORIDE INTOXICATION IN FISH: A REVIEW

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Abstract: A wide range of environmental and genetic factors cause fish to respond differently to given levels of fluorides, but they do display characteristic fluoride intoxication signs. Some of the variation can also be explained by postulating a chloride-fluoride excretion mechanism over the epithelial tissues. Such a mechanism would explain variations in toxicity correlated with different chloride concentration and the survival of natural populations of fish at fluoride concentrations which are lethal under laboratory conditions.

FLUORIDE IN THE AQUATIC ENVIRONMENT

Fluorides are widely distributed in the rivers, lakes, and seas of the world. The U.S. Geological Survey[10] reports that fluorides are common in the waters of the United States, especially in the west. Concentrations of 0.1 ppm are common and concentrations exceeding 1.0 ppm are not rare. Water samples from Walker and Pyramid Lakes in Nevada contain up to 13 ppm fluoride. The Madison and Firehole Rivers in Yellowstone National Park have fluoride concentrations ranging from 12-14 ppm. Kobayashi[5] reports 1.5 - 5.5 ppm of fluorides in wells in Japan. The natural thermal waters of New Zealand (pH 5-9) contain from 1-12 ppm of fluorides[7].

Most of the fluorides occur naturally. They are leached from fluoride, cryolite, apatite, and sedimentary phosphate rocks by precipitation and ground water. Pollution, both aerial and hydric also contribute fluoride to the aquatic environment.

Aquatic organisms would be expected to contain fluoride concentrations proportional to those in their environment. Neuhold and Sigler[9] reported mean concentration up to 1600 ppm in the bones of brown trout taken from the Madison River system. Lee and Nilson[6] recorded high concentrations of fluorides in the bones of canned salmon and mackerel. Similarly, Fisher[4] noted high concentrations of fluorides in fish meals used in the manufacture of prepared feeds.

FLUORIDE INTOXICATION

Signs of Fluorosis

Rainbow trout, carp and goldfish become apathetic and evidenced anorexia with the introduction of sodium fluoride to their medium. Goldfish suffering from anorexia lose weight[3], go through a period of violent movement which degrades into aimless wandering, and finally lose their equilibrium. The loss in equilibrium is accompanied by tetany and ends in death. Mucus secretion in all the fish tested increases with introduced fluoride. The increased mucus secretion is accompanied by proliferation of mucus-producing cells in the respiratory and integumentary epithelium[8].

The embryos of rainbow trout display much the same signs as adults when intoxicated with fluoride. Violent movement within the confines of the egg often cause the vitelline membrane to rupture prematurely. This phenomenon frequently is followed by death and an immediate coagulation of the yolk protein. Embryos that survive a fluoride-induced premature emergence have a high incidence of deformed spines[8].

Blood Changes

Changes occur in the blood of rainbow trout suffering from fluoride intoxication[1]. The level of total serum protein appears to drop in both trout and carp during intoxication[1,2]. The gamma and beta globulins specifically appear to change. Both serum alkaline phosphatase

activity and plasma magnesium levels increase, while calcium decreases with increasing fluoride concentrations.

Differentiation from Thermal Death

In thermal death, fish first show marked apathy then loss of orientation and equilibrium and finally gasping for breath, reduction of swimming ability, darkening of color, and then death. Occasionally there is limited muscular contraction just before death. After death the fish is limp. The difference in signs between fluorisis and thermal stress is that in the former, fish activity is much more intense in the early stages; in the final stage fluoride-induced death invariably results in some degree of tetany, generally quite intense and the skin does not discolor.

TOXIC LEVELS

The effects of fluorides in the environment, as reported by literature, all lead to the conclusion that fluorides above certain levels have a profound toxic effect on the physiology of animals.

The response of fish to moderate fluoride concentrations (1.5 to 5.0 ppm) is related to environmental acclimatization and is species dependent. It is difficult to assign specific values as toxic levels because so many factors in the environment, including the physiological state of the fish, the species, even the race or strain, govern the response of fish to intoxication. Various chloride concentrations in the medium affect the reactions of rainbow trout to fluorides[1]. Minnows subjected to simultaneously raised concentrations of chlorides and fluorides succumb to lower levels of fluorides than do fish subjected to only fluoride[1]. The amount of calcium in the medium also affects fish response. Higher than normal concentrations of calcium in the medium or the food tend to enhance the resistance of fish to fluorides[1].

[a]The LD_{50} is the dose lethal to 50 percent of t predetermined. Although the time is not prec between 48 and 240 hours.

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TOXIC LEVELS

The effects of fluorides in the environment, as reported by literature, all lead to the conclusion that fluorides above certain levels have a profound toxic effect on the physiology of animals.

The response of fish to moderate fluoride concentrations (1.5 to 5.0 ppm) is related to environmental acclimatization and is species dependent. It is difficult to assign specific values as toxic levels because so many factors in the environment, including the physiological st[illegible] of the fish, the species, even the race or strain, govern the response of fish to intoxication. Various chloride concentrations in the medium affect the reactions of rainbow trout to fluorides[4]. Minnows subjected to simultaneously raised concentrations of chlorides and fluorides succumb to lower levels of fluorides than do fish subjected to only fluoride[7]. The amount of calcium in the medium also affects fish response. Higher than normal concentrations of calcium in the medium or the food tend to enhance the resistance of fish to fluorides[5].

The time necessary for rainbow trout eggs to hatch decreases with an increase in the concentration of fluoride in the medium. It is concluded that larger fish, subjected to a given level of fluoride, tend to succumb last; that is, the larger the fish the more resistant they are to a given level of fluorides. The size of the fish has an effect on the length of the experiment only. No effect on the LD_{50}* or the sensitivity is apparent. The LD_{50} for rainbow trout, 10-20 cm in total length, in a medium low in both calcium and magnesium and at a temperature of 13C, is between 2.7 and 4.7 ppm. The LD_{50} for small carp falls between 75 and 91 ppm of fluoride when the same low calcium and magnesium concentrations are present[5].

Increasing the temperature of the medium increases the sensitivity of rainbow trout to fluoride intoxication. This relationship appears to be a function of the metabolic rate, which increases with temperature[6]. The relationship between temperature and metabolism is one of size and sensitivity of fish to fluorides. Small rainbow trout and small carp appear to be less resistant to fluoride intoxication than large ones.

Fish populations vary widely with respect to their ability to live in specific concentrations of fluorides. Healthy, growing populations of trout exist in the Firehole River in Yellowstone National Park where fluoride concentrations reach 14 ppm. The same is true of Pyramid and Walker Lakes in Nevada where the concentrations reach 13 ppm. Yet, rainbow trout that have been reared in low concentrations of fluoride display LD_{50} of approximately 3 ppm.

FLUORIDE UPTAKE

Fluoride uptake occurs in both soft tissues and bone in carp, goldfish and rainbow trout. The uptake in muscle tissue is highly variable and can be ascertained only between extremes. Osseous tissues are particularly good indicators of

* [illegible]D_{50} is the dose lethal to 50 percent of the experimental animals. The dosage schedule is [illegible]ermined. Although the time is not predetermined, experiments are generally terminated [illegible]ween 48 and 240 hours.

uptake of fluoride. Fish collected from the Madison River system showed a positive correlation between bone fluoride concentration and size of fish.

Goldfish subjected to chronic levels for 90 days accumulated fluoride at a rate similar to those for carp under acute levels[2,9]. The rate of uptake from the medium and its incorporation into the bone is by an apparent second order mode, suggesting active transport[6]. Fluoride concentration in the tissue is directly correlated with the amount of fluoride in the medium and with the duration of exposure. Fluorides accumulate more readily in osseous tissues[4]. When goldfish were subjected to low concentrations of fluorides (0.34-2.95 ppm) in soft water for 90 days there was an increase in bone fluoride concentration that correlated with both the median fluoride concentration and the duration of exposure[4].

TISSUE CHANGES

In one experiment, the epithelium of the gill filaments of rainbow trout displayed an increase in population density of mucus-producing cells from 0.31 at 0.0 ppm fluoride to 0.52 at 25.0 ppm fluoride. The epithelial tissue in the head region of rainbow trout fry subjected to two ranges of fluoride concentrations (0 to 25 ppm and 250 to 335 ppm) also indicated an increase in mucus cells. The tissue upon which these population density determinations were made was integumentary epithelial tissue located between the eyes[3].

Aside from the accumulation of fluorides in the tissues, other changes occur as well. The ultimobranchial gland (parathyroid function) hypertrophied in trout subjected to high levels of fluoride[1,3]. In the gill lamellae of goldfish, an edematous condition of the epithelium (described as a focal, non-specific, cytoplasmic enlargement, with the nuclei of the cells remaining unchanged) also occurs during fluoride intoxication[3]. If the mucus cells in the epithelium are assumed to act as a fluoride secretion mechanism, and if their proliferation is assumed to be a function of the fluoride concentration in the blood, the tendency would be toward an increased concentration of blood fluorides with increased concentration of medium fluoride.

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SEASONAL ABUNDANCE C
(MONOGENOIDEA) PARAS
Lepomis macrochirus (R

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Abstract: Nine species of Monogene
lected from *Lepomis macrochirus* in
from December 1967 to January
organism formed three distinct grou
the autumn and least abundant dur
triangularis and *Clavunculus bifurca*
the spring and least abundant du
seminolensis and *Cleidodiscus robus*
the summer but having a prewinte
Actinocleidus fergusoni, *Urocleidu*
nematocirrus.

Knowledge of seasonal abundanc
parasites will allow us to anticipat
tential disease problems and su
management procedures where fea
The object of this study was to
mine the seasonal abundance of an
cephalinaen parasites of one of the
important sport fish — the bluegill.

Seasonal abundance of the subf
Ancyrocephalinae has been report
only a few papers. Crane and M
found that on bluegill, *Lepomis m*
chirus Rafinesque, the *Urocleidus*
Mueller, 1934, population reached
levels in August and April and in
uary when the temperature was 8 C
highest populations of *Actinoc*
fergusoni Mizelle, 1938 occurred in
January and May. Meyer[5], utilizin
zootic case histories, felt that *Di*
gyrus populations were at the h
level in April but were common
the remainder of the spring and
summer and that *Cleidodiscus* on
was frequent from January to mid
mer.

response obtained from addition of Ca and perhaps other ions to the medium.

In previous work (*6*) with whole excised barley roots, $CaCl_2$ was found to have a much smaller effect upon respiration than that found here, while $CaBr_2$ and $CaSO_4$ were entirely without effect. This is probably a reflection of the greater need for Ca of cells close to the root meristem. In the case of excised barley roots the bulk of the tissue was composed of mature cells. There is of course also the possibility that the different responses to Ca found may be due to metabolic differences between the two species. Experiments with maize root sections further from the growing point are expected to illuminate this (*7*).

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5 September 1961

Chlorides Affect the Toxicity of Fluorides to Rainbow Trout

Abstract. Results of an experiment designed to test the effect of chloride ion concentration on fluoride toxicity to rainbow trout *(Salmo gairdnerii)* indicated that tempering fish to chloride reduced their response to fluoride.

On several occasions we have observed that fish collected for fluoride toxicity experiments varied in their group responses to the same concentration of fluoride. In every case these fish came from waters that had different chloride concentrations (*1*). Preliminary investigation indicated that the time required for the top minnow *Gambusia affinis* to succumb to a given concentration of fluoride increased when the normality of chloride was increased. This suggested that the chlorides had an effect on the toxicity of fluorides.

A 2 × 3 × 6 completely randomized factorial experiment, with rainbow trout as subject, was designed as follows: two replications of two qualities of tempering by three concentrations of chloride by six concentrations of fluoride were used. All combinations of these treatments were randomly selected and placed in 72 experimental units. Each unit consisted of five trout ranging from 4 to 7 inches and placed in a 20-gallon aquarium filled with 50 liters of softened water (the calcium and magnesium were reduced in an anion exchange column to a calcium concentration of less than 1 ppm and a magnesium concentration of less than 0.3 ppm). The tempering solutions were of the same quality softened water as the experimental units. The experiment was run for 120 hours at 7°C. All mortality occurred within the first 72 hours.

The fish were first placed into two 300-gallon holding tanks; in one the concentration of chloride (added in the form of sodium chloride) was 34 ppm. In the other it was 0 ppm. The fish were held in these aquaria for 48 hours before they were placed in the experimental tanks. The unit of measurement in the experiment was the number of fish in each unit that responded to the toxin.

Results of the experiment indicated that two factors had very significant effects on the response. The most striking was that tempering to chloride decreased the response of the trout to a given concentration of fluoride. The other was that increasing concentrations of fluoride bring about an increase in fish mortality (Table 1). Table 2 gives the statistical analysis of the results.

The LC_{50} (lethal concentration for 50 percent of the experimental subjects) was also found to differ significantly. 6 ppm elicited a response from 50 percent of the nontempered fish, while 22 ppm was required for the tempered fish.

Sensitivities of the fish to fluoride intoxication also differed significantly between the tempering treatments. The tempered fish responded less in terms of probits of response per unit increase in concentration of the toxin than the nontempered fish.

A number of authors have alluded to the existence of a specialized chloride secretion mechanism in fish (*2*). Copeland (*3*) states that chloride-secreting cells appear to respond to changes in chloride concentration in the blood. Similarly, we have found an increase in gill epithelium mucous cells when rainbow trout were subjected to increasing concentrations of fluoride (*1*).

The evidence presented by this experiment suggests that the chloride and

Table 1. Number of deaths of rainbow trout in response to combinations of various fluoride and chloride concentrations. The response indicated is the sum mortality in numbers of fish of 10 replications.

Fluoride concentration (ppm)	Deaths (No.)							
	Fish not tempered				Fish tempered			
	At Cl^- concn. indicated			Deaths, subtotal	At Cl^- concn. indicated			Deaths, subtotal
	0 ppm	3 ppm	9 ppm		0 ppm	3 ppm	9 ppm	
0	2	1	0	3	0	3	0	3
2	3	2	0	5	0	0	5	5
4	5	4	0	9	0	1	0	1
7	5	5	4	14	1	2	0	3
13	9	10	6	25	6	5	1	12
25	10	10	10	30	10	5	1	16
Totals	34	32	20	86	17	16	7	40

Table 2. Analysis of the variance of the responses shown in Table 1.

Source	D.F.	Sum of squares	Mean square	F-ratio
Replications	1	8.0000	8.0000	3.077
Tempering	1	29.3889	29.3889	11.303*
Chloride	2	14.2500	7.1250	2.740
Fluoride	5	113.6667	22.7333	8.743*
Tempering × chloride	2	0.3611	0.1805	0.069
Tempering × fluoride	5	16.4444	3.2889	1.265
Chloride × fluoride	10	15.0833	1.5083	0.580
Tempering × chloride × fluoride	10	25.3056	2.5306	0.973
Error	35	91.0000	2.6000	
Total	71	313.5000		

* Significant at the 99-percent level of confidence.

Sewage Works Journal

TABLE I.—Threshold Concentrations for Immobilization of Daphnia magna by Substances when Added to Lake Erie Water

Substance	Formula	Molarity*	P.P.M.*
Sodium acetate	$NaC_2H_3O_2$	<0.071	<5800
Sodium arsenate	Na_2HAsO_4	<0.00011	<20
Sodium arsenite	$NaAsO_2$	0.00007	9.1
Sodium benzoate	$NaC_7H_5O_2$	<0.0045	<650
Sodium borate	$Na_2B_4O_7$	≪0.0012	≪240
Sodium perborate	$NaBO_3$	≪0.000063	≪5.2
Sodium bromate	$NaBrO_3$	0.0014	210
Sodium bromide	NaBr	0.08	8200
Sodium carbonate	Na_2CO_3	<0.0040	<424—
Sodium bicarbonate	$NaHCO_3$	0.028	2350
Sodium chlorate	$NaClO_3$	0.040	4240
Sodium chloride	NaCl	<0.072	<4200
Sodium chromate	Na_2CrO_4	<0.0000020	<0.32
Sodium dichromate	$Na_2Cr_2O_7$	≪0.0000012	≪0.31
Sodium citrate	$Na_3C_6H_5O_7$	0.0032	825
Sodium cyanide	NaCN	<0.000069	<3.4
Sodium ferrocyanide	$Na_4Fe(CN)_6$	<0.0020	<600
Sodium fluoride	NaF	0.012	504
Sodium formate	$NaCHO_2$	<0.076	<5200
Sodium hydroxide	NaOH	0.0039	156
Sodium iodate	$NaIO_3$	≪0.00080	≪158
Sodium iodide	NaI	0.000022	3.3
Sodium nitrate	$NaNO_3$	0.059	5000
Sodium nitrite	$NaNO_2$	<0.00029	<20
Sodium nitroprusside	$Na_2Fe(CN)_5NO$	≪0.00080	≪210
Sodium oxalate	$Na_2C_2O_4$	0.0016	214
Sodium monobasic phosphate	NaH_2PO_4	≪0.013	≪1560
Sodium dibasic phosphate	Na_2HPO_4	≪0.00042	≪59
Sodium tribasic phosphate	Na_3PO_4	≪0.00032	≪52
Sodium salicylate	$NaC_7H_5O_3$	0.0091	1450
Sodium sulfate	Na_2SO_4	0.042	5960 SO4
Sodium bisulfate	$NaHSO_4$	0.0016	190
Sodium sulfide	Na_2S	0.00012	9.4
Sodium sulfite	Na_2SO_3	0.0035	440
Sodium bisulfite	$NaHSO_3$	<0.0014	<145
Sodium tartrate	$Na_2C_4H_4O_6$	<0.018	<3500
Sodium thiocyanate	NaSCN	<0.00014	<11.3
Sodium thiosulfate	$Na_2S_2O_3$	≪0.0033	≪520

* On the basis of the formula given.

that well fed *Daphnia magna* at 25° C. undergo their first ecdysis about twenty hours after their release from the brood chambers of the mothers. Some cast carapaces can be observed on the bottom of the experimental bottles at the sixteen hour observation when the animals are sixteen to twenty-four hours old. In Table I the concentrations preceded by either (<) or (≪) are not true threshold concentrations since the immobilization time-concentration curves for the salts designated had not reached their vertical asymptotes at forty-eight hours. The threshold concentration for any salt with (<) might be about nine-tenths of the value given if the trend of the curve were continued in each particular instance. The threshold concentration for a salt with (≪) might be as low as one-half the value given or less. The lack of (<) or (≪) does not mean, however, that the curve for any one salt might not be inflected were the observations continued for a longer period.

From a pollution standpoint the most

R78-7

TESTIMONY OF DR. MICHAEL BENDER

FEBRUARY 7, 1979

INTRODUCTION

Good day. My name is Michael Bender and I am Assistant Director and Head of the Division of Environmental Sciences and Engineering at the Virginia Institute of Marine Science in Gloucester Point, Virginia; and I am Vice President of Environmental Control Technology Corporation ("Encotec") in Ann Arbor, Michigan. Briefly, my academic background includes a B.A. from Southern Illinois University in 1961; a Master of Science in Fisheries from Michigan State University in 1962; and a Ph.D. degree in Environmental Sciences from Rutgers University in 1968. As my resume illustrates (a copy attached to this statement as Appendix 1), almost all of my professional training and work during the past 20 years has centered around the study and management of aquatic life and resources, with particular emphasis in determining the effect of pollution on aquatic life. The attached resume also includes chronological citations to my professional publications, which number about 30. As you can see, most of my professional experience and publications since 1960 are related to the subject of my testimony today.

shown you what changes are to be expected now, in view of the 1978 water quality data, following start-up of the new wastewater treatment plant on September 1, 1978. I have said that future aquatic life at E2 - E7, in about a year, will either equal or approximate aquatic life at E1, with an adjustment that reflects the gradient and flow of the ditch.

But all of this analysis has assumed that fluoride levels in the ditch are 1.4 mg/l -- the water quality limit in existing Rule 203 (f). However, the question in this proceeding for me to answer is: what effect, if any, would 5 mg/l of fluoride in the water have upon the benthos in the ditch? At this point in my testimony, I believe that you have almost all of the information you need for me to give you my expert opinion on that question. There are, however, two other items that need to be discussed.

First, the Board should know that there is little or no literature available on the potential effect of fluoride on benthic animals. To my knowledge, the only data that exists on this subject was collected by Encotec in Colorado from 1970 through 1974. Attached as Appendix 7 is a graph of this data which shows the effect of fluoride on the number of taxa (or species) of benthic animals in those Colorado waters. As you can see from this chart, there is no reduction in the number of species at up to 3.5 mg/l of

fluoride; and even at 4.5 mg/l, there is only about a 33% reduction.

The second item that the Board needs to know concerns hard and soft-water streams. The streams in which we collected the Colorado data were cold, soft-water trout streams -- a "soft-water stream" meaning that it is low in calcium and magnesium. The aquatic life in such streams is more sensitive to pollutants than in warm, hard-water streams. Hard water is high in calcium and magnesium. While to my knowledge there is no literature directly comparing the toxicity of fluoride on benthic animals in hard water versus that in soft water, there is information available on the toxicity of fluoride to fishes in hard water and in soft water, as Dr. Sigler will discuss later today. This literature shows that toxicity of fluoride to fishes decreases as the levels of calcium and magnesium increase -- as in hard water. Based on this information, it is reasonable for me to conclude that the same result would apply to the benthic life in a hard-water stream.

CONCLUSION

I have thus covered all the information needed by the Board for my expert opinion, and now we come to the ultimate question for my opinion. Would 5 mg/l of fluoride in GMC's ditch have a significant effect on the number of

INTERSPECIES RELATIONSHIPS IN ACUTE TOXICITY OF CHEMICALS TO AQUATIC ORGANISMS

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Abstract—Interspecies sensitivities of several species of aquatic organisms to chemicals categorized as nonpesticide organics, pesticides and metals were correlated using polynomial regression analyses. All species analyzed—fresh and saltwater fish, invertebrates and algae—responded similarly to the toxicity of nonpesticide organics. Different groups of organisms responded differently to the pesticides. Different species of fish of the same family responded almost identically to the toxicity of pesticides, whereas fishes of different families responded similarly, but to a lesser degree. No relationship existed between the acute sensitivities of a fish and an invertebrate. A significant correlation was determined in acute sensitivities to metals between bluegill and fathead minnows and between bluegill and *Daphnia magna*. The mode of toxicity of metals seems to be the same among species, although the degree of toxicity may differ.

Keywords—Pesticides Organics Metals Toxicity Aquatic organisms

INTRODUCTION

Determining acute toxicity is the first requirement when assessing the toxicological hazards associated with a potential pollutant in the aquatic environment. Any comprehensive hazard assessment will require acute toxicity data, using a variety of species occupying several trophic levels. The U.S. Environmental Protection Agency (EPA) [1] lists several species recommended for acute toxicity tests and subdivides them into four basic categories: freshwater vertebrates, freshwater invertebrates, marine and estuarine vertebrates and marine and estuarine invertebrates. When estimating the acute toxicity of a pesticide intended for use in the United States, a warm-water fish, a cold water fish and a freshwater invertebrate must be used if the product is intended for outdoor application. Saltwater fish, shrimp and crab acute toxicity tests are required if the pesticide is intended for direct application to the estuarine or marine environment [2]. When estimating the acute toxicity of an industrial effluent, the EPA [3] recommends that species indigenous to the receiving water be used. The recommended base ecotoxicity data set for chemicals subject to premanufacture notification under the

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The rationale for testing a variety of aquatic species is clear. Insufficient data exist that elucidate reliable interspecies relationships regarding sensitivity to toxicants. To maximize the protection of all biotic components of the aquatic environment, it is advisable to determine the susceptibility of as many component representatives as is economically and reasonably possible. Such an approach should not be discouraged, considering the current developmental stages of the science of aquatic toxicology and the art of hazard assessment. However, should some definitive interspecific toxicological relationships exist, defining such relationships would greatly enhance the hazard assessment process when the reliability of certain data is suspect, the certain necessary data have not been obtained because of time, economic or other constraints and/or the susceptibility of a critical indigenous species has not been determined due to its unavailability or collecting and handling sensitivity.

A few interspecific toxicological relationships have been investigated. Patrick et al. [5] compared the acute susceptibility of fish, diatoms and snails to several toxins. No consistent trends in susceptibility were determined. However, had the confidence intervals been presented for the TLm's used, it might have become apparent that the acute sensitivities of the three groups of organisms were similar for many of the toxins. Kenaga and Moolenaar [6] compared the acute sensitivity of fish, daphnids, aquatic vascular plants and algae to thousands of chemicals of heterogeneous structures. They found that animals were more sensitive indicators of toxic effects than were vascular plants or algae. These authors concluded that toxicity data for fish and daphnids should be sufficient to protect algae and aquatic vascular plants. Maki [7] found the chronic sensitivity of the be an attractive alternative to life cycle toxicity tests with fish.

Since different types of toxicants have different modes of toxic action, it would be naive to presume that, should some interspecific toxicological relationships exist, they would apply to all chemicals. In the present study, various interspecies comparisons were made, with the acute toxicities of chemicals catagorized into three generic categories. (a) Nonpesticide organic compounds: These compounds were selected from the U.S. EPA list of priority pollutants that were not formulated specifically to contain pesticidal properties. This group consisted of organic compounds formulated to exhibit specific toxicological properties and which are lethal to certain groups of organisms at low doses. (b) Metallic Materials which occur naturally in the aquatic environment but which also have anthropogenic sources.

MATERIALS AND METHODS

Acute toxicity tests used for nonpesticide organic compounds with warm- and cold-water fish were performed at [illegible] Bionomics, Aquatic Toxicology Laboratory

Table 1. Preliminary nonpesticide organic compound LC_{50} values used for the comparison of species sensitivities

Test material	LC_{50} (mg/L) Bluegill	Rainbow trout
A	23	6.3
B	120	100
C	2,500	1,100
D	0.13	0.20
E	0.41	0.74
F	300	430
G	[illegible]	4.3
H	1.6	0.76
I	310	160
J	2.9	0.17
K	1,000	1,000
L	1,000	770
M	4.3	4.2

tions [1]. All other acute toxicity data used for nonpesticide organic compounds were obtained from LeBlanc [8], Buccafusco et al. [9], Heitmuller et al. [10], and the EPA [11] (Table 2). Only compounds that were water soluble at the concentrations tested, as indicated by Buccafusco et al. [9], were used in the comparisons. Pesticide data were obtained from the four sources listed for nonpesticides, as well as Johnson and Finley [12] and the EPA [13] (Table 3). Metals data were obtained from the EPA [11] and LeBlanc et al. (personal communication) (Table 4). For metals for which numerous LC_{50} values were available for a single species, the average LC_{50} values in soft water (< 100 mg/L $CaCO_3$) were used.

All LC_{50} values, in mg/L, were converted to logarithms for statistical comparison. Comparisons were made by polynomial regression analysis using a Hewlett-Packard model no. 9815A programmable calculator. Significance was determined at $p \leq 0.05$ and $p \leq 0.01$. Forty-eight-hour LC_{50} values

RESULTS

Nonpesticide organic compounds

Warm-water fish vs. cold-water fish. Because of differences in metabolic rates at different temperatures, it is generally presumed that the sensitivities of warm-water and cold-water fish may vary significantly for toxicants when the mode of toxicity involves metabolic pathways. Figure 1 shows the correlation between the LC_{50} values for bluegill (*Lepomis macrochirus*) and for rainbow trout (*Salmo gairdneri*) tested with 15 nonpesticide organic compounds. A highly significant ($p = 0.0$) straight-line correlation existed between the acute sensitivities of these two species. Further, the sensitivities of the two species were nearly the same. Rainbow trout exhibited a slightly greater sensitivity within the range of LC_{50} values tested. The relationship between the acute sensitivities of rainbow trout and bluegill to nonpesticide organic compounds was as fol-

Table 2. Nonpesticide organic compound LC_{50} or EC_{50} values used for the comparison of species sensitivities

Test material	LC_{50} or EC_{50} (mg/L)*					
	S. capricornutum	*D. magna*	*L. macrochirus*	*S. costatum*	*M. bahia*	*C. variegatus*
1,2-Dichlorobenzene	91.6	2.44	5.59	44.2	1.97	9.11
4-Chlorophenol	5.01	4.06	3.83	3.27	29.7	5.35
Dibenzofuran	0.18	0.23	0.41	0.18	0.12	0.25
Diphenyl ether	1.7	0.67	1.7	0.80	0.71	2.4
N-Docosane	>500	>530	>530	>500	>500	>500
Sodium fluoride	272	338	>530	181	23.3	>500
Methylethyl ketone	>500	>519	>530	>500	>402	>402
Toluene	>433	313	12.7	>433	56.3	366
Nitrobenzene	44.1	27.0	42.6	10.3	6.68	58.6
4-Nitrophenol	4.19	21.9	8.28	7.37	7.17	27.1
2,4,6-Trinitrophenol	11.7	84.7	167	62.7	19.7	134
Tetrachloroethylene	>816	17.7	12.9	509	10.2	39
[illegible]	4.95	15	6	0.40	0.59	1.77
2,3-Dinitrotoluene	1.6	0.66	0.93	[illegible]	87.8	75
Ethylbenzene	>438	73.0	155	>438	24.4	17.9
Bromoform	117	45	29.3	112	[illegible]	331
Methylene chloride	>662	224	224	>662	[illegible]	12.3
1,1,2,2-Tetrachloroethane	136	9.15	11.3	6.44	5.02	2.36
1-Chloronaphthalene	1.03	1.60	2.27	1.13	0.37	[illegible]

Bull. Environm. Contam. Toxicol. 24, 684-691 (1980)

Acute Toxicity of Priority Pollutants to Water Flea (*Daphnia magna*)

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Industrially used chemicals are potential sources of contamination of the aquatic environment either through normal usage, disposal, or accidental spillage. In order to properly evaluate the potential hazards of these materials to the aquatic environment, toxicity tests must be conducted with aquatic organisms. Data derived from toxicity tests are used by the United States Environmental Protection Agency to develop water quality criteria for such chemicals. The purpose of this study was to determine the acute toxicity of selected priority pollutants to the water flea (Daphnia magna). Water flea are commonly used organisms in aquatic toxicity tests, and their susceptibility to contaminants in the aquatic environment tends to be representative of freshwater zooplankton.

MATERIALS AND METHODS

All chemicals tested were purchased from commercial chemical suppliers and had a minimum purity of 80%. The chemicals were tested on an active ingredient basis and concentrations are reported as milligrams (mg) of test material per liter (L) of diluent water.

Daphnia magna (≤24 hours old) used in these toxicity tests were from laboratory stocks cultured at EG&G, Bionomics. Water used to culture the organisms used in the initial 15 tests was deionized reconstituted well water having a total hardness of 72 ± 6 mg/L as $CaCO_3$ and a pH of 7.0 ± 0.2. Subsequently, culture water was reconstituted according to U.S. EPA (1975) to a total hardness of 173 ± 13 mg/L as $CaCO_3$ and a pH of 8.0 ± 0.2, to improve conditions for test organisms.

Procedures used in these acute toxicity tests were based on protocols in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975). Diluent water used in these tests was of the same quality as previously described for water used to culture these animals. At the initiation of all tests, the dissolved oxygen concentration of diluent water was

TABLE 1 -- continued

Test Substance	LC50 (mg/L) 24-hour	LC50 (mg/L) 48-hour	No discernible effect conc. (mg/L)
diethanolamine	170 (-)[c]	55 (44-68)	<24
n-dibutyl ether	32 (26-36)	26 (21-33)	4.6
diphenyl ether	1.4 (1.1-1.9)	0.67 (0.11-1.1)	0.41
n-docosane	>530	>530	<68
sodium fluoride	680 (-)	340 (280-410)	110
methylethylketone	>520	>520	<70
α-pinene	68 (24-190)	41 (27-62)	8.8
styrene	27 (20-35)	23 (18-29)	<6.8
biphenyl	27 (19-48)	4.7 (3.6-5.9)	<2.2
dibenzofuran	7.5 (4.4-13)	1.7 (1.1-2.7)	0.28

[a]Diluent water had a mean hardness of 72 mg/L as $CaCO_3$.

[b]95 percent confidence interval.

[c]Confidence interval was not calculatable.

O = LC_{50} < 1 ppm

ACKNOWLEDGEMENTS

The author extends sincere appreciation to Donald Surprenant, Brian Robinson, Mitch Ziencina and Mary Hawes for their technical assistance in the completion of this study.

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STUDIES ON THE ACUTE TOXICITY OF FLUORIDE ION TO STICKLEBACK, FATHEAD MINNOW, AND RAINBOW TROUT

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Abstract: We have studied the acute toxicity of fluoride ion to Gasterosteus aculeatus, Pimephales promelas, and juvenile Salmo gairdneri. LC50 values varied with species and (due to precipitation) initial water hardness. Exposure to elevated fluoride levels in water resulted in increased blood fluoride levels in Salmo gairdneri.

Introduction

Interest in environmental fluoride ion and fluoride salts has long been spurred by observance of differing effects of fluoride, depending on exposure level. While the toxicity of high levels and the benefits of trace levels appear well established (Underwood, 1971), the question of what level is safely tolerable in the environment remains less clearly delineated (U.S. Environmental Protection Agency, 1980b). As with other potential pollutants, fluoride's effects in aqueous systems and on aquatic life have been of particular concern. Our laboratory has carried out a number of static bioassay studies intended to define the acute toxicity of fluoride ion to sticklebacks (Gasterosteus aculeatus), fathead minnows (Pimephales promelas), and juvenile rainbow trout (Salmo gairdneri) (Holsen et al., 1985). As will be discussed below, our results suggest that fluoride may not be as acutely toxic to fish as certain earlier studies concluded. There are indications of a threshold toxicity effect in all three species. Our results also support the suggestions of others (Herbert and Shurben, 1964; Vallin, 1968; Pimentel and Bulkley, 1983) that the observed protective effect of high water hardness may be due to the precipitation of insoluble calcium fluoride from hard water. Finally, measurements of blood fluoride levels in rainbow trout exposed to fluoride indicate a modest increase in blood fluoride at sublethal levels, but markedly higher concentrations in the blood of fish exposed to fluoride levels near the LC50. Following a summary of our results, we will discuss our findings in the context of data previously reported by other researchers, and of regulatory concerns.

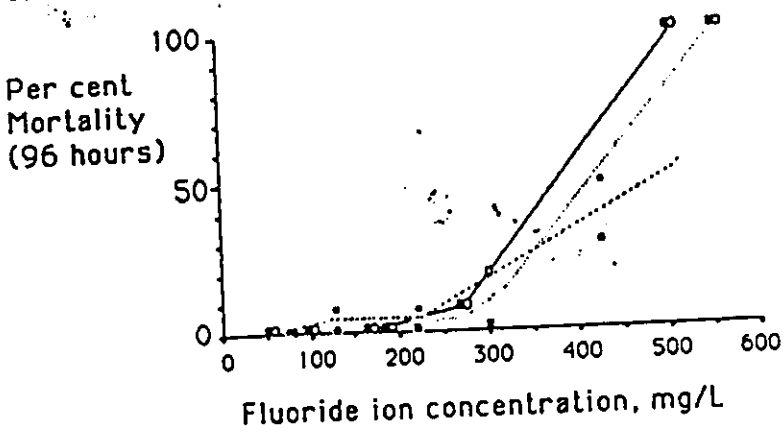
Static Bioassays

In Table 1, we summarize our static bioassay conditions, and the 96-hour LC50 values derived. All fish used were obtained from Alex Fish Co., San Rafael, CA. Sticklebacks and fathead minnows used were typically less than one gram in size; trout were generally less than three grams. Bioassays were run in duplicate, using ten individuals per ten liter tank. Except as indicated, initial fluoride levels were not replenished during the course of an experiment. Bioassay water was dechlorinated San Francisco tap water, which generally has a hardness below 50 mg/L (as $CaCO_3$), and a fluoride ion level of ca. 0.5 mg/L. Fluoride

ion concentrations were adjusted with reagent grade sodium fluoride, and hardness was adjusted using reagent grade calcium sulfate and magnesium sulfate. Fluoride ion concentration was monitored by means of an Orion fluoride electrode, and hardness was measured by EDTA titration. Combinations of high fluoride and moderate to high hardness caused rapid precipitation of finely divided solid, which spectrographic analysis indicated to consist of calcium and magnesium salts. In two of the fathead minnow experiments, fluoride levels were restored after precipitate formation. Because of the incompatibility of high fluoride and high hardness, there was no attempt to restore hardness levels after precipitate formation.

LC50 values were determined graphically; data was plotted on log-probit paper, with test concentrations entered on the log scale and per cent mortality on the probit scale (American Public Health Association, 1981). From our results, any protective effect of water hardness appears slight, and is probably due to loss of fluoride ion to precipitation. Figures 1, 2 and 3, graphing mortality directly against fluoride level, appear to show a threshold toxicity effect as exposure concentrations approach the LC50 value for each species. While trout and fathead minnows appear more sensitive to fluoride ion than do sticklebacks, the overall range of 96-hour LC50 values observed in our studies (180 to 460 mg/L, depending on species and conditions) varied by a factor of only 2.55.

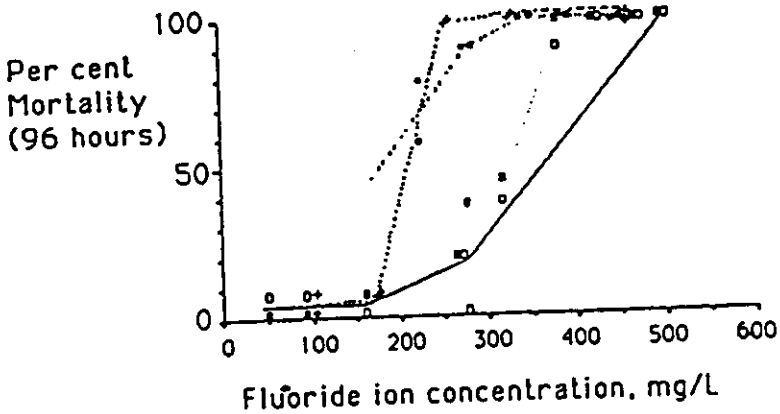
Figure 1: Fluoride ion toxicity to stickleback, Gasterosteus aculeatus



Notes on test conditions:

Temperature 20° C, hardness 78 mg/L--●,○
Temperature 20° C, hardness 146 mg/L--■,□
Temperature 20° C, hardness 300 mg/L--▲,△

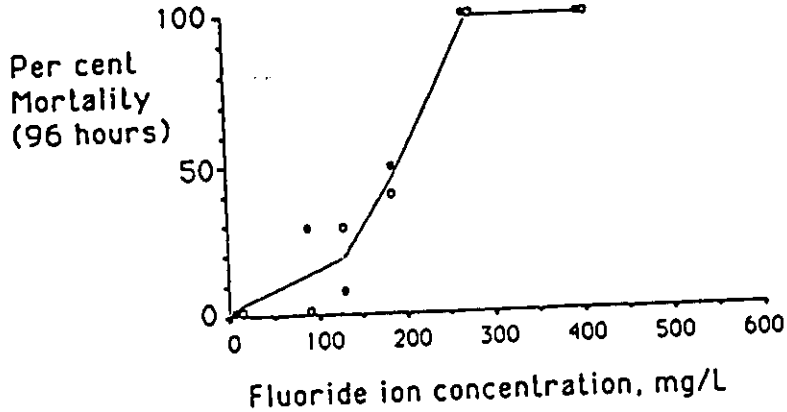
Figure 2: Fluoride ion toxicity to fathead minnow, Pimephales promelas



Notes on test conditions:

Temperature 16-20° C, hardness 20-48 mg/L -- •,o
Temperature 15-19° C, hardness 10-44 mg/L -- •,o
Temperature 20° C, hardness 92 mg/L -- •,•
Temperature 20° C, hardness 256 mg/L -- •,•

Figure 3: Fluoride ion toxicity to rainbow trout, Salmo gairdneri



Notes on test conditions:

Temperature 15° C, hardness 23-62 mg/L -- •,o

Blood Fluoride

For measurements of blood fluoride in trout, somewhat larger individuals (3.4-5.1 grams) were used than in the 96-hour bioassays, to facilitate collection of sufficient blood for fluoride measurements. Tanks were set up at several fluoride concentrations, with the LC50 chosen as the highest concentration. At intervals, living fish were removed from the test tanks, wiped dry, and their tails were amputated with a scalpel. Microhematocrit tubes were used to collect 10-20 µL of blood from the vein paralleling the backbone. A microtechnique, in which the fluoride electrode was placed flat against a 1-cm filter paper disk moistened with sample, was used to measure fluoride levels in 10 µL of fish blood after mixing with 10 µL of ionic strength adjustment buffer ("TISAB").

Our blood fluoride results are summarized in Table 2. High mortality prevented measurements beyond one day at the 200 ppm exposure, but extended survival at lower concentrations permitted measurements over a 10-day period. While the data are limited, they indicate a leveling-off of blood fluoride levels within a few days. These results supplement earlier studies by others, which found that prolonged exposure of fish to fluoride results in accumulation of fluoride both in bone and in soft tissue (Neuhold and Sigler, 1960; Wright and Davison, 1975; Wright, 1977; Milhaud, El Bahri, and Dridi, 1981). One study has suggested that although trout may be relatively sensitive to fluoride as compared with other fish, adaptation may also be possible; a case of wild trout successfully adapted to 14 mg/L of fluoride was cited (Sigler and Neuhold, 1982). In some, but not all, marine organisms, prolonged exposure to moderate fluoride levels appears to be tolerable (Hemens and Warwick, 1972; Hemens, Warwick, and Oliff, 1975; Milhaud, El Bahri, and Dridi, 1981).

Discussion

A range of widely divergent LC50 values has been reported for fluoride in rainbow trout and other species of fish. While the reported variations may predominantly reflect variables such as exposure time, precipitation due to water hardness, fish size, differences in strains of fish tested, and test temperature, it may also be that the conclusions of certain early studies cited below cannot be confirmed. In 1960, Neuhold and Sigler determined a 24-day LC50 for fluoride of 2.7-4.7 mg/L in rainbow trout; in 1961, Angelovic et al. measured a 10-day LC50 of 5.9-7.5 mg/L for the same species. Soon thereafter, a 21-day rainbow trout LC50 value of 8.5 mg/L (in soft water) was reported (Herbert and Shurben, 1964). However, in 1968, Vallin reported that rainbow trout in hard (320 mg/L, as $CaCO_3$) water survived 100 mg/L of fluoride for 21 days; formation of a precipitate of calcium fluoride was also mentioned. Much more recently, a study of the effect of water hardness on fluoride toxicity in rainbow trout (Pimental and Bulkley, 1983) found 96-hour static LC50 values ranging from 51 mg/L to 193 mg/L, depending on hardness. Our own 96-hour static LC50 value of 200 mg/L for rainbow trout, measured at an intermediate initial hardness level,

corresponds roughly to conditions under which Pimental and Bulkley obtained an LC50 value of 128 mg/L. It is of interest to note that although the earliest studies indicate much higher toxicity for fluoride than we found, some studies also suggest threshold toxicity effects for fluoride ion (Herbert and Shurben, 1964; Wright, 1977).

Discussions of fluoride toxicity are complicated by the status of fluoride both as a beneficial trace element (Underwood, 1971; McKee and Wolf, 1977; National Academy of Sciences (U.S.A.), 1972; U.S. Environmental Protection Agency, 1980a) and as a potential toxin in larger doses (Windholz et al., 1983; McKee and Wolf, 1971; California Department of Health Services, 1984; U.S. Environmental Protection Agency, 1980b). Imputed levels of hazard, such as the California "STLC" (soluble threshold limit concentration) of 180 mg/L for leachable fluoride, were set at least partly on the basis of the earliest studies, which indicated a higher fish toxicity level for fluoride than more recent experiments. Drinking water standards, which are relatively low (ca. 1 mg/L), may also have tended to influence regulatory views of potential hazards due to elevated fluoride ion concentrations in water. The available data suggest that a uniform consensus about the maximum safe level of fluoride ion for fish in natural waters of varying hardness has not yet been achieved.

Table 1: Summary of Fish Bioassay Results

Species	96-hr LC50, mg/L	Fluoride Replenished to maintain level?	Initial hardness (mg $CaCO_3$/L)	Initial pH	Final pH	Temp., °C
Stickleback	340	no	78	7.4	7.7-7.9	20°
Stickleback	380	no	146	7.4	7.5-7.9	20°
Stickleback	460 (1)	no	300	7.4	7.6-7.9	20°
Rainbow trout	200	no	23-62	7.4	7.7-8.0	15°
Fathead minnow	315	no	20-48	8.0-8.2	7.9-8.0	16-20°
Fathead minnow	315	no	10-44	7.5	7.7-8.0	15-19°
Fathead minnow	180 (1)	yes	92 (2)	7.4	7.7-7.8	20°
Fathead minnow	205	yes	256 (3)	7.5-7.6	7.6-7.7	20°

(1) by extrapolation

(2) Due to rapid precipitation, this hardness was maintainable only in the control tank. Hardness dropped to 10 in test tanks within a few hours, staying high only in the control tank.

(3) Within a few hours, actual hardness dropped to 12 in the 400 ppm fluoride tank, and to 75 in the 100 ppm fluoride tank.

Table 2: Rainbow trout (Salmo gairdneri) blood fluoride levels

Exposure Time	Blood level in Control fish	Blood level, fish in 75 ppm tank	Blood level, fish in 150 ppm tank	Blood level, fish in 200 ppm tank
4 hr	<2 (4)	-	-	3.0 ± 1.1 (4)
1 day	<0.5 (4)	6.0 ± 4.6 (5)	7.8 ± 4.4 (5)	17.4 ± 9.6 (5)
2 days	<0.5 (1)	4.7 ± 4.5 (5)	9.0 ± 7.7 (4)	-
3 days	<0.5 (4)	6.7 ± 2.3 (5)	3.0 ± 0.9 (5)	-
6 days	<0.5 (3)	4.1 ± 2.3 (5)	2.2 ± 0.5 (4)	-
8 days	0.5 (3)	3.8 ± 1.1 (5)	-	-
10 days	<0.5 (3)	3.4 ± 1.5 (5)	-	-

Source of fish: Alex Fish Co., San Rafael, CA
Weight range: 3.4-5.1g
Length: 6.8-8.4 cm
Numbers in parentheses indicate the number of individuals sampled.
Indicated uncertainties are standard deviations.

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Environmental Chemistry

Short Communication

INFLUENCE OF WATER HARDNESS ON FLUORIDE TOXICITY TO RAINBOW TROUT

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Abstract — Static bioassays were conducted to determine effects of water hardness on toxicity of sodium fluoride to rainbow trout (*Salmo gairdneri*). Ninety-six hour LC_{50} values increased from 51 to 193 mg/L [F] as water hardness levels rose from 17 to 385 mg/L $CaCO_3$. Tests of chronic toxicity at different water hardness levels are needed before fluoride standards for aquatic life, such as fish, can be set.

Keywords — Fluoride Toxicity Water hardness Acute bioassay Rainbow trout

INTRODUCTION

Considerable information has been collected in the past on the toxicity of fluoride to freshwater fish. Unfortunately, levels that produce adverse effects are still difficult to predict because toxicity is influenced not only by the usual factors such as size [1], species [2] and physiological state of the individual fish, but also by the chemistry of the water [3]. Tolerance of fish to fluoride is increased by low temperature, low concentrations of chloride ion and high levels of calcium hardness in the water [3–7]. Because of these complexities, criteria for fluoride in wastewater often fail to reflect the influence of important environmental factors on toxicity. For example, recommended upper limits of fluoride in water for domestic use range from 1.4 mg/L [F], when the annual average of maximum daily air temperature is 27 to 33°C, to 2.4 mg/L [F], when air temperatures average 10 to 12°C [8]. The rationale for setting a sliding scale for water for domestic use based on temperature is that people and animals tend to drink more water when the climate is warm. Temperature should also be considered when standards are set for aquatic life such as fish because high temperature increases their metabolic rates and brings about faster absorption of fluoride [4]. The much larger range in measured toxic effects to fish caused by variation in water hardness is not considered in the criteria [8], even though the relationship has been known for more than four decades [5]. In soft water, the 20-d LC_{50} for rainbow trout (*Salmo gairdneri*), 10 to 20 cm in length, is 2.7 to 4.7 mg/L [F] [9]. Hence, water hardness should be considered

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when numerical standards of fluoride are set for fish. And, because fluoride standards are not set for aquatic wildlife, agriculture or industry in many states, there may be a tendency to impose the rigorous standard for domestic use of water on these and other users at a time when water is becoming scarce and its multiple use imperative.

The purpose of our study was to determine acute toxicity of different concentrations of fluoride at different levels of water hardness to rainbow trout, a species common to cool waters of the United States. Data offered here may provide the background for future long-term chronic toxicity studies on the influence of water hardness, which are needed so that safe fluoride standards for fish can be established.

MATERIALS AND METHODS

The rainbow trout used in this experiment were hatched and reared at the Hotchkiss (Colorado) National Fish Hatchery and were certified disease-free. They were transferred to the Utah Water Research Laboratory in Logan, Utah, by air shipment when they were about 30 mm long (swim-up stage). At the Laboratory, the fish were held in 300-liter flow-through tanks at 8°C in water from the Logan River at a natural photoperiod; they were fed commercial dry trout food for 90 d. Fish used in the experiments were 58.7 ± 4.3 mm ($\bar{x}$ ± SD) long and weighed 1.8 ± 0.5 g.

Exposure to fluoride occurred in 19-liter standard bioassay jars filled to a depth of 29 cm with 15 liters of Logan City water, which had been dechlorinated and deionized (≤ 1 × 10^6 ohm) and had salts added to obtain the desired pH and hardness (Tables 1 and 2). The fish were acclimated to a test temperature of 12°C and a specific water hardness in an 833-liter static, covered tank. A photoperiod of 14 h light and 10 h darkness was maintained. The temperature was raised 1°C/d, and water hardness was raised or lowered by replacing half the water in the acclimation tank each day with water of the desired hardness. After 4 d, the water was 94% of the desired hardness and the temperature was at 12 ± 0.1°C. The fish were left undisturbed for two more days before they were transferred to bioassay jars. Fish were not fed during the 6 d of acclimation or the 4 d of exposure.

Fluoride was added to the jars in the form of reagent-grade sodium fluoride (Mallinkrodt). Sodium fluoride was first dissolved into a superstock solution of 20 g/L NaF ([F] = 9.05 g/L; NaF weighed to 0.1 mg, diluted in a 500-ml dilution flask) and then pipetted into jars in the appropriate amounts to give a logarithmic series of concentrations, 0.25 logarithm apart. Water samples for fluoride analysis were taken before the fish were placed in the jars and again after the 96-h test. Samples were swamped with total ionic strength adjustment buffer (TISAB) [10] and analyzed with an Orion fluoride probe (model no. 94-09), Corning Calomel reference electrode and a Corning no. 130 pH/mv meter. Accuracy of this method was approximately 84 to 113%.

Six fish were placed in each bioassay jar (loading rate = 0.2 g of fish per liter of

Table 1. Quantities of reagent-grade chemicals required to prepare recommended reconstituted fresh water and the resulting water qualities[a]

Water Hardness	Salts required (mg/L)				pH[b]	Hardness[c]	Alkalinity[d]
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl			
Very soft	12	7.5	7.5	0.5	6.4–6.8	10–13	10–13
Soft	48	30.0	30.0	2.0	7.2–7.6	40–48	30–35
Hard	192	120.0	120.0	8.0	7.6–8.0	160–180	110–120
Very hard	384	240.0	240.0	16.0	8.0–8.4	280–320	225–245

[a]From ref. 13.
[b]Approximate equilibrium pH after aeration and with fish in water.
[c]Total concentration of calcium and magnesium salts expressed as mg/L $CaCO_3$ [10].
[d]Quantitative capacity to neutralize acid to a designated pH, expressed in mg/L $CaCO_3$ [10].

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ardness[c]	Alkalinity[d]
10–13	10–13
40–48	30–35
80–180	110–120
80–320	225–245

10].

Table 2. Actual quality of the reconstituted freshwaters used in this experiment (analyzed according to ref. 10)

Water hardness	Ions analyzed (mg/L) Ca^{2+}	Mg^{2+}	Cl^-	pH[a]	Hardness[b]	Alkalinity[c]
Very soft	4.7	1.5	1.1	7.2	17	11
Soft	11.3	7.5	2.5	8.3	49	36
Hard	32	25	4.0	8.3	182	139
Very hard	63	75	8.5	8.7	385	397

[a]Measured after 18 h aeration and with no fish in the water.
[b]Total concentration of calcium and magnesium salts expressed as mg/L $CaCO_3$ [10].
[c]Quantitative capacity to neutralize acid to a designated pH, expressed in mg/L $CaCO_3$ [10].

water) after the toxicant had been added. There were three jars of fish per concentration (total of 18 fish) and five concentrations plus a control for each of the four levels of water hardness. Jars were randomly placed equidistant from each other in three 833-liter covered water baths, six jars per bath. Water baths were maintained at 12 ± 0.1°C on a 14 h light and 10 h dark photoperiod, with a 15-min "dawn" and "dusk." Data for fish from each concentration were pooled to determine 96-h LC_{50} values [F]. The criterion of effect was death, defined as the fish floating upside down and not operculating. LC_{50} values were estimated from both initial calculated and measured concentrations of fluoride. We calculated the 96-h LC_{50} values and 95% confidence intervals using the moving average methods of Thompson [11] and Weil [12]. Throughout the experiment, tests were conducted as recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms [13].

RESULTS

Since LC_{50} values obtained from calculated and measured concentrations of fluoride were similar (Table 3), results are discussed in terms of measured concentrations.

The 96-h LC_{50} for rainbow trout exposed to fluoride in very soft water (17 mg/L $CaCO_3$) was 51 mg/L (Table 3). Increasing the water hardness to 49 mg/L $CaCO_3$ doubled the LC_{50} to 128 mg/L. Further increase of water hardness to 182 mg/L $CaCO_3$, however, only increased the LC_{50} to 140 mg/L. When the water hardness was doubled again to 385 mg/L $CaCO_3$, the LC_{50} increased to 193 mg/L, a value significantly higher than the other LC_{50} values.

When the LC_{50} values were plotted against water hardness (Fig. 1), a definite trend of decreasing fluoride toxicity with increasing water hardness was observed. This trend followed the logarithmic curve of LC_{50} expressed by the formula LC_{50} (mg/L) $= -51.73 + 92.57 \log_{10}$ (water hardness in mg/L $CaCO_3$). The correlation coefficient (r) value of this relationship was 0.95. At the highest water hardness (385 mg/L $CaCO_3$), fluoride began to precipitate out, presumably as CaF_2 [6,9]. The amount of fluoride that precipitated varied from 9 to 30%, depending on the concentration of fluoride. The percentage was determined by subtracting the final from the initial concentration and dividing by the initial concentration. Recalculation of the 96-h LC_{50} for very hard water with the final instead of the initial concentrations of fluoride still in

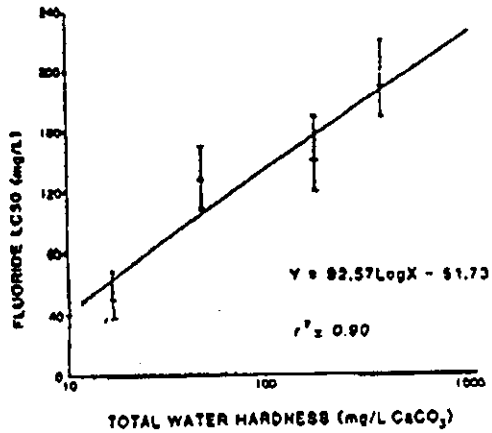

Fig. 1. The 96-h LC_{50} values for fluoride in relation to water hardness.

Table 3. Summary of calculated and measured fluoride exposure concentrations (mg/L)

Calculated fluoride concentration (mg/L)	Hardness level (mg/L)[a]											
	17			49			182			385		
	Measured fluoride		No. dead fish	Measured fluoride		No. dead fish	Measured fluoride		No. dead fish	Measured fluoride		No. dead fish
	Start	End		Start	End		Start	End		Start	End	
0	0.02	0.02	0	0.51	0.20	0	0.08	0.03	1	0.12	0.15	0
10	9.9	9.1	1				36.3	33.8	0	43	39.3	0
18	17.9	17.0	4	34	35.2	0	54.1	63.2	0	90.8	63.6	0
32	31.4	30.0	5	62.3	67.1	0	96.5	99.1	3	172	121	5
56	56.1	53.4	7	114	122	8	172	171	13	320	257	18
100	97.4	96.4	17	172	194	16	316	296	18	577	473	18
180				336	346	18						
320										193		
560				128			140			(167–223)		
LC_{50}(M)[b]	51			(108–150)			(117–167)			202		
	(38–68)						142			(176–231)		
LC_{50}(C)[c]	52			117			(119–169)					
	(39–70)			(108–127)								

[a]Number of dead rainbow trout of a total of 18 exposed at each concentration, and 96 h LC_{50} values (95% confidence interval in parentheses) for fluoride at four levels of water hardness.
[b]Based on measured hardness.
[c]Based on calculated hardness.

solution gave a 96-h LC_{50} of 136 mg/L (confidence limits, 121–153 mg/L). This LC_{50} was essentially the same as that for the next lower water hardness (Table 3), and reflected the amount of fluoride precipitated out.

DISCUSSION

Results of these experiments confirm reports of other investigators that acute effects of fluoride are influenced by water hardness. Direct comparison of LC_{50} values with those from other studies is not possible because of the different methods used to report toxic effects. It is evident, however, that rainbow trout survived concentrations of fluoride in hard water that were lethal in softer water. The protective effect of calcium carbonate hardness is evidently produced through both chemical and physiological processes. In hard water, high concentrations of fluoride are reduced by formation and subsequent precipitation of CaF_2 [6,9]. Sigler and Neuhold [3] reported that fluoride also forms stable complexes with calcium in the blood and bone. Hence, a reservoir of calcium in the water surrounding the fish tends to compensate for this loss of calcium and thereby delays toxic effects of fluoride on the organism.

The desirability of establishing fluoride standards for fish on the basis of water hardness becomes evident when one examines the hardness of surface waters in Utah. Of 56 stream gauging stations in Utah where chemical data were collected in the 1979 water year, water at only two stations had a hardness level below 200 mg/L [14]. Mean $CaCO_3$ water hardness was 1,010 mg/L, and the median hardness for the 56 stations was approximately 370 mg/L. Based on our acute toxicity findings, and with other factors being equal, an estimated 50% of rainbow trout fingerlings could tolerate 4-d exposures to concentrations of 193 mg/L [F] in at least half the waters tested in Utah. At only one station would mortality higher than 50% be expected in 140 mg/L [F]. Obviously, however, 50% mortality in 96 h is unacceptable for protecting fish. Until studies can be conducted to determine safe chronic exposure concentrations, the use of a general application factor based on a fraction of the 96-h LC_{50} level [8] seems appropriate. The application factor for substances that accumulate in the fish body (fluoride is concentrated mainly in boney tissue [5]) is 0.05 times the 96-h LC_{50}. Hence, recommended interim maximum chronic exposure levels range from 2.5 mg/L [F], at a water hardness of 17 mg/L, to 9.6 mg/L [F] at a hardness of 385 mg/L. Long-term studies are now needed to determine how well this application factor reflects the maximum acceptable toxic concentrations for fluoride in waters of varying hardness.

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[a]Number of dead rainbow trout of a total of 18 exposed at each concentration, and 96-h LC_{50} values (95% confidence interval in parentheses) for fluoride at four levels of water hardness.
[b]Based on measured hardness.
[c]Based on calculated hardness.

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Effect of fluorides on survival and reproduction of *Daphnia magna*

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Fluorides occur naturally in water and usually are found in areas that have been subject to recent volcanic activity. In natural waters, concentrations of 0.1 mg/L are common, but concentrations that exceed 1.0 mg/L are not unusual. Certain industries, such as aluminum, steel, phosphate, and glass production, are also significant sources of fluorides for aquatic eco-systems.[1,2]

The toxic and health effects of fluorides have been studied extensively over the past several decades, mostly with regard to their effects on mammals and especially on humans.[1] Microorganisms, aquatic invertebrates, and fish have received only minor attention, and very little information is available on the chronic effect of fluorides on aquatic organisms.[2] Currently, the only data on long-term effects of fluorides was published by Neuhold and Sigler[2] who conducted experiments with rainbow trout embryos and fry at 15.6°C (60°F) for 825 hours. Long-term bioassays with warm water fish and freshwater invertebrates were not performed; therefore, safe concentrations for fluorides to protect aquatic life were not established. *Daphnia magna* was chosen to determine the critical concentrations of fluorides because it is sensitive to pollutants, has a short life cycle, and is an important fish food organism; furthermore, the widespread use of Cladocerans in other toxicity studies allows comparison of results.

There are no federal ambient water quality criteria for fluorides; however, several states have established their own standard for this contaminant. This paper provides some of the baseline data that can be used to formulate meaningful water quality criteria.

MATERIALS AND METHODS

Experimental animals. Daphnids (*Daphnia magna*) used for acute and chronic toxicity experiments were from in-house cultures originally obtained from a commercial biological supply house. The stock cultures were maintained in 5-L aquaria that contained gently aerated (20 cc/min) hard water (170 mg/L as $CaCO_3$) using 16-hr photoperiods at temperatures of 19 to 21°C. The experimental animals were fed 3 times a week (Monday, Wednesday, and Friday) with a water suspension of a mixture of trout chow, alfalfa, yeast, and commercial fish food. The water in the aquaria was changed approximately every 10 days, and if necessary, the culture was thinned. Only neonates less than 24 hours old were used as starting organisms in all toxicity experiments.

Environmental conditions. Dilution water used for stock cultures and experiments was reconstituted hard water (169 mg/L as $CaCO_3$) prepared according to method 801D in Standard Methods[3] (Table 1). Double distilled water was prepared in an all-glass still, and reagent-grade chemicals were used to pr the reconstituted water. The toxicant was reagent-grade so fluoride (NaF); concentrations were selected on the basis logarithmic expansion.

Temperatures were constantly monitored and maint during the experiments with a constant-temperature ba ±1°C as described by Peltier[4]. The temperatures selecte acute toxicity testing were 15, 20, and 25°C; chronic exp were performed at 20°C.

Five chemical analyses were performed at the beginnin end of each acute test and 10 times during the chronic t each water change. Hardness was determined by EDTA metric method 314B.[3] Alkalinity was measured by pot metric titration (method 403[3]) with 0.02 *N* HCl to pH 4. was measured electrometrically. Dissolved oxygen was mined by the membrane electrode method 421F.[3] Fluoride concentration was measured with a selective ion electrod adding a total ionic strength adjustment buffer (method 4 Total fluoride additions were weighed to 0.1 mg and re as F^-.

A 16-hr photoperiod was used: a 10-hour fluorescen period at 65 ft-c, preceeded and followed by a 3-hour pe incandescent light at 4 ft-c.

Daphnid food was prepared by mixing 3 g trout chow commercial fish food, 2.6 g dried yeast, and 0.5 g dried with 500 mL double distilled water in a blender at high for 7 minutes. The mixture was refrigerated for 1 hour, t 300 mL of the suspension were decanted and frozen in polyethylene bottles for future use, and the sediment w carded. In addition, green algae (*Selenastrum capricor* were used to feed the daphnids. Algal cultures were esta and maintained using an original (UTEX-1648) strain o from the University of Texas at Austin.[5]

The formation of complexes with polyvalent ca and several other factors can cause significa differences in toxicity results.

Testing. Methods for measuring acute toxicity gener lowed the guidelines of Peltier.[4] Acute toxicity was dete from 48-hr static tests using 20 daphnids in 4 replicates concentration of toxicant. Five neonates were placed in four 100-mL glass beakers filled with 80 mL of solut feeding or aeration was used. The measured endpoint w tality, as determined by lack of movement of antennae abdominal claw on gentle prodding.

Table 1—Composition of reconstituted water.

Salts added to double distilled water		Water quality measured—median value (95% confidence interval)	
$NaHCO_3$	192 mg/L	pH	8.14 (8.13–8.15)
$CaSO_4 \cdot 2H_2O$	120 mg/L	Hardness	169.3 (167.8–170.9) mg/L as $CaCO_3$
$MgSO_4$	120 mg/L		
KCl	8 mg/L	Alkalinity	110.8 (106.1–115.4) mg/L as $CaCO_3$

The interim guidelines of the U. S. EPA[5] were followed for chronic toxicity tests. Chronic toxicity was determined from a 21-day renewal static test, using 20 daphnids for each concentration of fluoride; one neonate was placed in each of twenty 100-mL beakers filled with 80 mL of solution. Water was changed on Days 3, 5, 7, 10, 12, 14, 16, 18, and 20. Feeding consisted of 0.4 mg of prepared food suspension plus approximately 10 million cells of *Selanastrum capricornutum* following each water change. No aeration was used. Observations included mortality, time to the first brood, number of young per brood, and the number of "eggs" and neonates produced by the adult daphnids. Live neonates were counted by individually pipetting the organisms out of the test beaker; the number of eggs produced by individual females was established by pipetting all debris from the test beaker, placing this debris on a petri dish, and examining the contents under a dissecting scope at 10× power. The egg count was based on self-contained eggs aborted by the adult, and eggs contained in the shed carapace, dead embryos and live neonates.

Statistical methods used for calculation of the LC_{50} values were "log concentration versus percent mortality method" and the "moving average angle method" as described by Peltier and Weber.[6]

RESULTS

Acute test. A summary of fluoride concentrations and *D. magna* mortality for the acute tests are given in Table 2. Measured concentrations affecting the survival were greater than 124 mg/L F^- (average concentration at start and end of test) at 15°C, greater than 93 mg/L F^- at 20°C, and greater than 52 mg/L F^- at 25°C. A similar temperature-related response was detected when LC_{50} values were calculated using the moving average angle method. The 48-hour LC_{50} for measured fluoride concentrations were 350, 247, and 180 mg/L for temperatures 15, 20, and 25°C, respectively. The LC_{50} values for total fluorides were 10% to 13% higher than those obtained for measured (ionic) fluorides (Table 3).

The graphical method (log concentration versus percent mortality method) produced lower LC_{50} values for the 15°C temperature than the moving average angle method; the values obtained for 20 and 25°C were greater. The values calculated by the moving average angle method for total and ionic fluorides are graphically depicted in Figure 1. The noted relationship between increasing fluoride toxicity and increasing water temperature could be characterized by the following equations:

$$LC_{50} = (6.93 - 0.065T) \text{ for total fluorides} \quad (1)$$

$$LC_{50} = (6.84 - 0.066T) \text{ for ionic fluorides} \quad (2)$$

where

T = temperature, °C.

Measured temperatures for the acute tests had median values of 15.0, 20.0, and 24.9°C. The 95% confidence interval (C.I.) for temperature in all cases was within ±0.1°C of the median values. Dissolved oxygen was within 95 to 100% of saturation in all concentrations.

Chronic test. In the 3-week exposure test, survival and reproduction of daphnids exposed to varying fluoride concentrations (Table 4) was studied (Table 5). Impairment in reproduction was observed in measured fluoride concentrations greater than 26 mg/L. A concentration of 35 mg/L of fluoride reduced the neonate production (average number of neonates per adult during the 21 days) to 44% of the control. The average number of live young dropped by more than 98% at 49 mg/L F^- and daphnids exposed to 84 and 142 mg/L F^- did not produce any live neonates.

A stimulating effect occurred at the lowest fluoride concentration (26 mg/L); production of neonates exceeded the number of young observed in the control. Also, the females exposed to this concentration produced young more rapidly than daphnids

Table 2—Fluoride concentrations and daphnid mortality (acute test).

Total fluoride concentration, mg/L	Test at 15°C: Measured ionic fluoride concentration, mg/L Start	Test at 15°C: Measured ionic fluoride concentration, mg/L End	Test at 15°C: Percent mortality	Test at 20°C: Measured ionic fluoride concentration, mg/L Start	Test at 20°C: Measured ionic fluoride concentration, mg/L End	Test at 20°C: Percent mortality	Test at 25°C: Measured ionic fluoride concentration, mg/L Start	Test at 25°C: Measured ionic fluoride concentration, mg/L End	Test at 25°C: Percent mortality
0	0	0	0	0	0	0	0	0	0
63	—	—	—	—	—	—	55	50	0
100	—	—	—	96	90	0	86	84	15
158	123	126	0	144	136	10	136	134	25
251	210	210	20	224	210	15	230	240	25
316	286	290	35	284	290	80	300	280	100
398	374	350	100	380	376	100	—	—	—
447	416	410	100	—	—	—	—	—	—

Table 3—LC_{50} values for acute tests.

	LC_{50}, mg/L F^- (95% confidence interval)	
	Log concentration versus percent mortality method[a]	Moving average angle method[a]
Total fluoride basis	15°C: 335 20°C: 284 25°C: 220	15°C: 385 (361–413) 20°C: 279 (285–302) 25°C: 201 (169–239)
Ionic fluoride basis	15°C: 304 20°C: 251 25°C: 200	15°C: 350 (329–376) 20°C: 247 (224–272) 25°C: 180 (146–221)

in the control water (Figure 2). Reproductive enhancement v also initially recorded at 35 mg/L F^- where the total number live young exceeded reproduction in the control on Day however, after this brief increase, reproduction at this conc tration was impaired. Alternately, the average total product of eggs exceeded the control in all concentrations except mg/L F^-. In higher fluoride concentrations, however, most these eggs did not hatch or were aborted in the form of eggs embryos (Figure 3).

DISCUSSION AND CONCLUSIONS

Several authors suggest that temperature may be an import modifier of toxicity; however, there is no general or consist effect of warm or cold water on toxicity. Depending on the spe

Table 4—Chemical analyses of test water at 20°, chronic test—median (95% confidence interval).

Total F^- concentration (mg/L)	Ionic F^- concentration (mg/L)	Hardness (mg/L) median value (95% confidence interval)	Alkalinity (mg/L)	pH
0	0	181.3 (168.5–194.1)	126.3 (120.0–132.6)	8.16 (8.06–8.27)
25	26.1 (24.4–27.8)	181.6 (174.4–188.8)	139.6 (135.0–144.2)	8.19 (8.10–8.28)
40	35.5 (33.8–37.2)	158.7 (146.5—170.9)	135.4 (131.5–139.3)	8.18 (8.10–8.25)
63	49.0 (46.5–51.5)	133.8 (124.7–142.9)	135.8 (131.7–139.9)	8.17 (8.08–8.25)
100	83.8 (81.0–86.5)	114.2 (109.2–119.3)	136.5 (131.7–141.3)	8.14 (8.06–8.22)
158	141.6 (137.8–145.4)	101.6 (98.2–105.0)	138.6 (135.5–141.7)	8.17 (8.08–8.27)

Table 5—Reproduction data and mortality at 20°C (chronic test).

Total fluoride concentration, mg/L	Ionic fluoride concentration, mg/L (95% confidence interval)	Coefficient of variation of ionic fluoride concentrations (%)	Number of eggs (Average number per adult)[a]	Number of live neonates (Average number per adult)[a]	Total number of mortalities during test[b]	Hatchab rate[c] ('
0	0	—	129.2	122.3	0	94.7
25	26.1 (24.4–27.8)	3.3	153.5	130.0	0	84.7
40	35.5 (33.8–37.2)	2.5	143.7	53.6	0	37.3
63	49.0 (46.5–51.5)	2.6	131.1/178.4	1.7	3	1.3
100	83.8 (81.0–86.4)	3.2	174.4	0	0	0
158	141.6 (137.8–145.4)	1.4	117.8	0	1	0

[a] Average number calculated on basis of 10 adults with/without adjustment for mortalities.
[b] Mortalities at 63 mg/L occurred on Days 10, 14, 14; at 158 mg/L on Day 21.
[c] Hatchability rate is the number of live neonates divided by number of eggs.

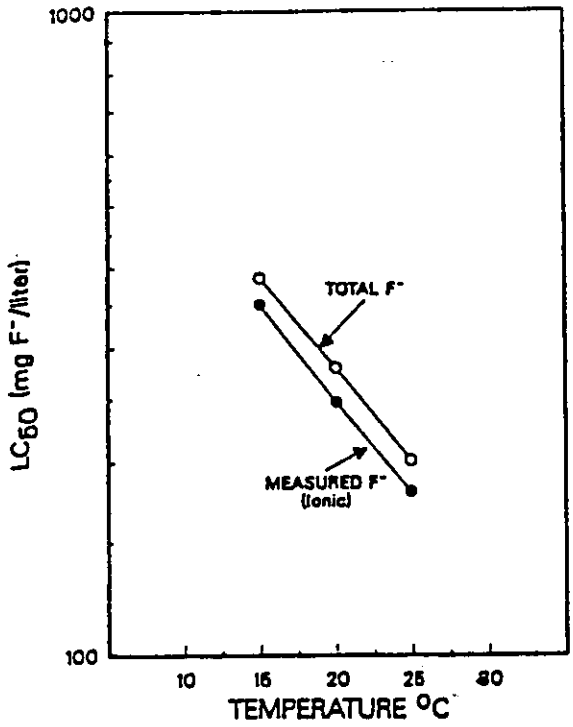

Figure 1—LC_{50} versus temperature (acute tests).

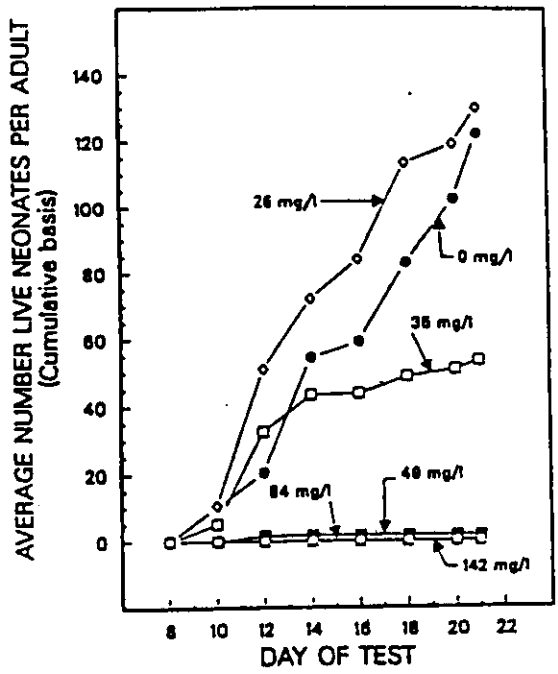

Figure 2-Live neonate production at different fluoride concentrations (chronic test).

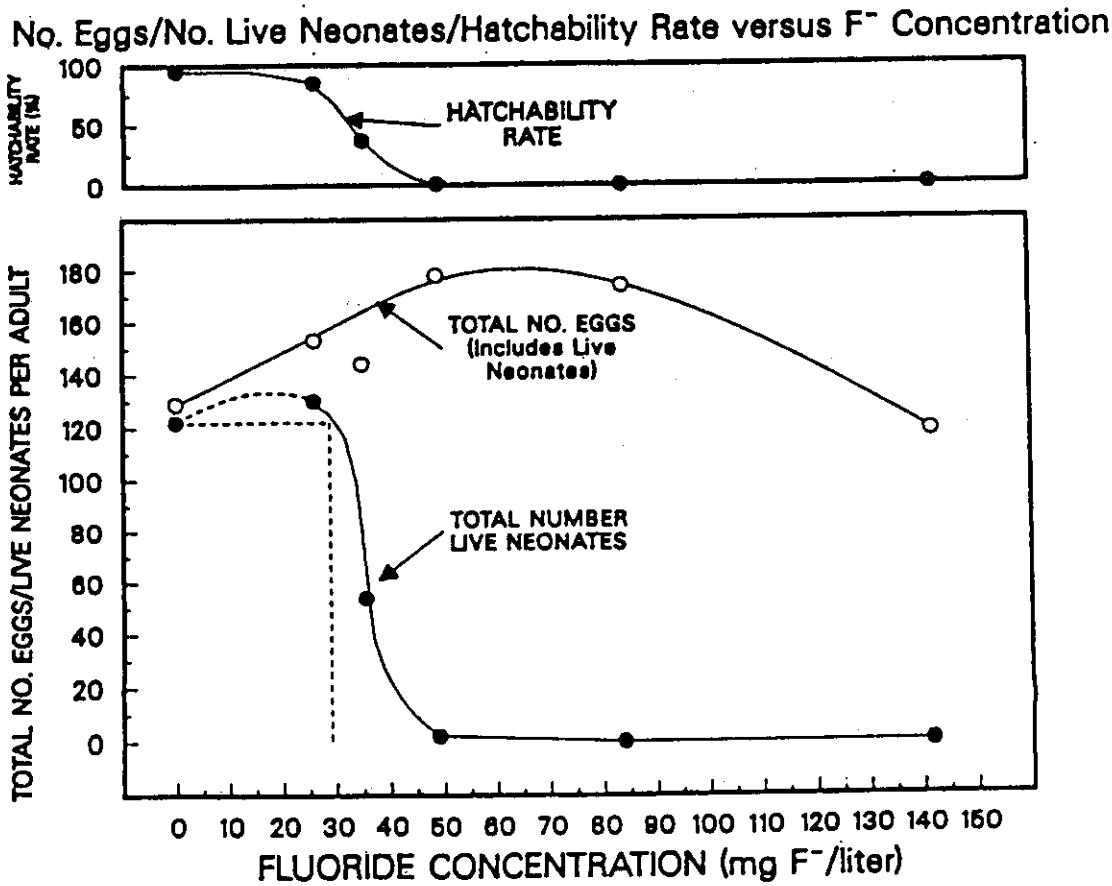

Figure 3—Reproduction of *D. magna* at 20°C (chronic test).

and pollutant, fish in warmer water may be more, less, or equally tolerant.[7]

The results of the acute test confirm the idea expressed by Angelovic *et al.*[8] that the toxicity of fluorides to aquatic organisms may increase with temperature; however, because the authors did not provide a final conclusion which would express the mathematical relationship between the water temperature and the effect of fluorides on freshwater animals, a quantitative comparison to these results cannot be made.

The model developed in this study shows that there is a simple exponential relationship between the toxicity of fluorides and temperature in hard water. This effect was possibly produced through physiological processes, as at higher temperatures the increase in metabolic rate may have resulted in increased uptake of fluoride by the daphnids.

Several authors have noted the reduction in measured fluorides relative to hardness.[9,10,11] Figure 1 illustrates the difference between introduced (total) and measured (ionic) fluoride levels. It is essential to differentiate between total and measured fluoride content, especially at higher fluoride concentrations. Factors such as formation of complexes of fluoride anion with the polyvalent cations of the hard water constituents, elevated activities, solubility limits of fluoride salts, and variations in temperature can cause significant differences in toxicity results.

The fate of fluoride contaminants as they enter a river or stream of a given hardness is important from a pollution control viewpoint, and total as well as measured fluoride concentrations are important. The measured or ionic form of fluoride concentration is important from the ecological viewpoint because it is immediately available for uptake by organisms.

Chronic testing of *Daphnia magna* in hard water at 20°C indicates that the presence of fluoride anions has a multiple effect on the reproductive system. Increases in fluoride content up to 65 mg/L F^- (Figure 3) stimulate egg production by increasing amounts up to about 150% of that of the control. Conversely, increasing fluoride concentrations reduce the hatchability rate continuously with a sharp decrease around 34 mg/L. Neonate production equivalent to that of the controls was established at 29 mg/L F^- which is, in effect, a "break-even" point between increased egg production and reduced hatchability (Figure 3). The chronic-to-acute ratio calculated from this "safe" concentration and the corresponding acute value for hard water at 20°C was 0.12; the application factor[12] calculated on the basis of maximum acceptable toxicant concentration (MATC) was established to be between 0.11 and 0.14.

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Effects of Sodium Fluoride on Carp and Rainbow Trout

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ABSTRACT

The symptoms of acute fluoride intoxication in carp and rainbow trout include lethargy, violent and erratic movement, and death where there is partial or complete muscle contraction. Excessive mucus production associated with an increase in mucous cells in the epithelium of the head region and the gills also occurs. Changes in the electrophoretic pattern of the serum proteins in carp blood are evident. There is an increase in the fluoride concentration of the bones and a hypertrophy of the ultimobranchial gland. Rainbow trout embryos display initial symptoms similar to adult fish, including lethargy, violent movement, and tetany. Rupturing of the chorion by the embryo is attributed to violent movement during the onset of intoxication. Lethal doses and the sensitivities of fish to the toxin are dependent on several variables including size of fish, temperature of medium, and calcium and chloride concentration of the medium. It was demonstrated that there is an uptake of fluoride in muscle tissue, cancellous bone, and skeletal bone, and that uptake was increased when there was a rise in the fluoride concentration of the medium. The uptake of fluoride by the bone was via a second-order reaction and is assumed to be an enzymatic process. Proliferation of mucous cells in the epithelium of the gills and the head region is postulated to be instrumental in the excretion of fluoride from the body, and is considered an effective defense mechanism against fluoride intoxication. Hypertrophy of the ultimobranchial gland is thought to result from a deficiency in calcium induced by the effects of fluorides. The uptake of fluoride by the bone is a defense mechanism against fluoride intoxication and is postulated to occur with the elimination of fluoride from body circulation. This fluoride forms a stable mineral complex.

INTRODUCTION

The fluoridation of domestic water supplies to control dental caries has raised a number of questions, including the effect of fluorides on aquatic plants and animals. Fluorides in domestic water supplies may be derived from alcohol distilleries, phosphate plants, metal refining plants, and other industries which discharge effluents or gases containing fluorides into the atmosphere and surface waters. Aerial pollutants are leached from the atmosphere by rain and snow (Adams *et al.*, 1952; Gabovich, 1952) and into the aquatic environment. Another source of aquatic pollution is fluoride leached from superphosphates which have been applied as soil fertilizer.

Fluorides are introduced into the aquatic habitat from such natural sources as fluorite, apatite, cryolite, and sedimentary phosphate rocks. Gabovich (1952) reports that an estimated 15,117 tons of fluorides per year are carried by streams of the Ukranian Territory. The United States Geological Survey (1957) has shown that fluorides in water are common especially in western United States. Most of these waters had concentrations in the magnitude of tenths of a part per million, but a significant portion contained more than 1 p.p.m.

Areas of recent volcanic activity also add significant amounts of fluorides to water. Kobayashi (1951) reports wells in Japan which contain from 1.5 to 5.5 p.p.m. fluorides. Our surveys indicate that hot springs and geysers of Yellowstone National Park contain from 25 to 50 p.p.m. fluorides. Firehole River and Madison River in Yellowstone National Park contain from 1 to 14 p.p.m. fluorides. Water samples from Walker and Pyramid Lakes in Nevada contain as much as 13 p.p.m. fluorides.

Literature concerning the toxicity of fluorides to fish is rare. Ellis (1937) reports 1,000 p.p.m. fluoride killed goldfish, in hard water, in 60 to 102 hours. In soft water mortality time at 1,000 p.p.m. ranged from 12 to 29 hours. DeRoos[1] reports that mortality attributable to fluorides occurred on goldfish in 120 p.p.m. fluoride. Lee and Nilson (1939) found that canned salmon and mackerel held high concentrations of fluorides in bones. Fisher (1951) indicates some prepared feeds containing fish meal contain relatively high concentrations of fluorides.

[1]DeRoos, Carolyn Clapp. (1957) The effects of sodium fluoride on the weight gain and gills of the common goldfish. M.S. Thesis, Utah State University, Logan, Utah. 40 pp.

In order to assess the effect of fluorides on fish, two fresh-water teleosts were selected as experimental subjects: the carp, *Cyprinus carpio* Linnaeus, and the rainbow trout, *Salmo gairdneri* Richardson. Both fish are eurhaline. Between them they have a distribution in the Western States which covers most of the ecological niches from near-alpine to sea level. In addition, both species have sport and commercial importance.

MATERIALS AND METHODS

The equipment for trout experiments consisted of eighteen 20-gallon aquaria located in refrigerated rooms which could be varied from 40° to 60° F. Each aquarium had both fluorescent and incandescent light. A submerged air line capped with a 1-inch carborundum stone supplied 30 cubic inches of air per minute. Surface velocity was approximately 0.1 foot per second with a complete surface turnover every 30 to 40 seconds. Carp experiments were conducted in two 270-gallon and one 150-gallon tanks. Lighting and aeration were accomplished in the same manner as for the 20-gallon aquaria.

Rainbow trout eggs used for toxicity experiments were supported by a plastic screen within a vertical plastic cylinder open at both ends. The bottom of the cylinder was vented, and the air stone placed on top provided the necessary energy for a vertical circulation of water over the eggs. Experiments were initiated with eggs which had been held in 52° F. water for 20 days after fertilization. During experiments the eggs were in total darkness.

Fluoride concentrations were maintained by adding soluble sodium fluoride to the water until it tested at the desired level. Since all the acute intoxication studies were done in soft water (calcium and magnesium were removed in a cation exchange column) only an insignificant portion of the fluoride remained associated and suspended in the medium. The amount taken up by the fish and that which may have been complexed with biological excretions also appeared to be insignificant since no difference could be discerned between the fluoride concentration at the start and end of each experiment.

The water used was from Logan City, Utah, municipal water supply which after softening had the following analysis:

Material	p.p.m.
Total dissolved solids	251-297
Magnesium	0.0-0.3
Calcium	0.5-1.5
Potassium	4.3-5.6
Sodium	97-103
Sulfates	5.0-9.0
Bicarbonates	185-212
Carbonates	0.0
Chloride	5.0-12.0
Fluoride	0.10-0.20

Population densities

The proper densities at which to hold fish in the aquaria were determined empirically. Aquaria were initially overpopulated, oxygen consumption and the ammonia production were measured, and the effects on the fish noted. Numbers were then reduced until no significant change in the levels of oxygen and ammonia was detectable and until the fish displayed no abnormal antagonism to each other. These numbers were used as the maximum population densities for toxicity experiments.

The maximum allowable concentration of accumulated ammonia was 0.4 gram per square centimeter of surface (6.7 grams per liter) for carp and 0.1 gram per square centimeter of surface (3.33 grams per liter) for trout. Carbon dioxide was not accumulated, and there appeared to be no social antagonism at these levels. Temperature ranges were 60°-65° F. for carp and 50°-55° F. for trout. The actual rates of introduction of fish for the toxicity experiments were usually at 50 percent of the determined maximum allowable rates. This approaches the densities recommended by Doudoroff *et al.* (1951).

Rainbow trout eggs were held at densities of approximately 0.0037 gram per square centimeter of surface (0.25 gram per liter). No change in the oxygen concentration was observed. The ammonia concentration after 424 hours was less than 1 p.p.m. The temperatures at which rainbow trout eggs toxicity experiments were conducted ranged from 45° to 60° F. and bracketed the optimum temperature for development and survival.

Temperatures at which the toxicity experiments on rainbow trout were conducted were from 53° to 57° F. This temperature range agrees closely with the upper optimum growth

temperatures for *Salvelinus* sp. as reported by Brown (1957). Temperatures at which carp toxicity experiments were conducted were held from 60° to 70° F., a range which approached the optimum temperature for growth in carp as reported by Sigler (1958).

Chemical analysis of tissue

Both rainbow trout and carp were dissected to separate skeletal muscle, cancellous bone (represented by the opercular assemblies), and skeletal bone (represented by the vertebral column). After dissection the tissues were autoclaved for 2 minutes at 15 pounds to coagulate the protein. The samples were then wrapped in aluminum foil and frozen until analyzed. Since the samples were small with respect to the total amount of fluoride present, a micro-quantity fluoride determination technique was used (Nielsen, 1958). The values thus obtained had a precision of 5 percent in terms of gammas of fluoride.

This technique was also used to determine fluoride in water when samples were suspected of containing interfering ions. If interference was not a problem, water was analyzed by the application of the A.D.H.A. Megregian-Maier modifications as presented in *Water and Sewage Analysis* (Hach Chemical Company, Ames, Iowa). Determination of ammonia, oxygen, and carbon dioxide followed the techniques presented in Ellis *et al.* (1948). Blood samples were taken from fish by making a diagonal excision across the caudal peduncle, immediately posterior to the anal fin, to sever the caudal vein and artery. The blood was collected in a 3-inch plastic centrifuge tube and allowed to coagulate for 3 hours at room temperature. It was then centrifuged at 2,500 revolutions per minute for 20 minutes. The clear serum was syringed from the centrifuge tube, placed in 2-inch glass tubes which were sealed with polyethylene sheeting, and stored for periods not exceeding 3 days at 40° F.

Serum protein separations were made in a Spinco paper electrophoresis chamber for 14 hours. After development, the paper strips were analyzed in a Spinco Analytrol. The values obtained were in terms of percentage of total serum protein.

Histological examination

Tissue samples taken for histological preparation included the gills of rainbow trout and carp, the entire body of rainbow trout sac fry, and the esophagi of rainbow trout. The techniques followed were those of Keyes and Willmer (1932) for the preparation of gills to detect chloride-secreting cells. The population densities of epithelial mucous cells were determined by counting their occurrence in 30 adjacent cells of a randomly selected gill filament, or in a medial section of integumentary epithelium of the head region. The esophagi were prepared for the determination of fluoride effects on the thyroid and ultimobranchial body which is reputed to have parathyroid function in trout (Brown, 1957).

Experimental design and analysis

The toxicity experiments used two designs. The first involved a gross division of concentrations of fluoride in magnitudes of 100 p.p.m. from 0 to 500 p.p.m. added as sodium fluoride. The concentrations were arranged at random within the physical plant. The same number of fish were used in each concentration. This design was used to determine at which concentrations complete mortality could be expected. The second design included concentrations which ranged between those at which no mortality and complete mortality occurred. These concentrations were 0, 2, 4, 7, 13, and 25 p.p.m. for the rainbow trout.

The responses of carp and of rainbow trout eggs were adequate in the gross ranges and no further subdivision of the concentrations was necessary. These experiments used an analysis of the probit responses and followed the techniques presented by Finney (1952) and Goulden (1952). Both the sensitivity of the fish to toxin and the L.C. 50 (lethal concentration to 50 percent of the fish) were estimated at the 95 percent confidence level. Rainbow trout egg toxicity experiments followed a randomized block design in which the temperatures were blocked and the concentrations distributed at random within the blocks. The analyses from these experiments also followed the probit analysis technique.

The first calcium-fluoride relationship experiment was conducted on the basis of two simple factorial designs. The first was a 2 by 6 in which the fluoride concentrations were 0 and 25 p.p.m., and the calcium concentrations were 0, 1, 2, 3, 4, and 5 p.p.m. The fluoride was added as sodium fluoride and the calcium as calcium fluoride. The fluoride of calcium was added to the water in the situation where the amount of calcium required was less than the solubility of calcium fluoride. Additional increments of fluoride were added as sodium fluoride.

The second experiment was a 3 by 6 factorial in which the fluoride concentrations were 0, 7, and 13 p.p.m., and the calcium concentrations were 0, 2, 4, 7, 13, and 25 p.p.m. The fluoride was added as sodium fluoride and the calcium as calcium hydroxide. The constituents were dissolved with the aid of carbon dioxide as dry ice, which was added to the water to provide a slight change in the pH. After the constituents were dissolved the water was agitated to drive off the excess carbon dioxide. This changed the pH from 8.0 to 7.9. This slight change in pH was attributed to a slight persistence of carbonic acid in solution. These experiments involved a probit analysis of response to the log metameter of the ratio of fluoride to calcium. The results of the two experiments were combined in the analysis. In all of the toxicity experiments the units of measurement included the response of the fish, or the eggs, in terms of percentage mortality.

To determine the relationship of fish size to fluoride concentration in terms of mortality, another simple factorial was used. Carp in this experiment were arranged in length groups. The design was a 3 by 5 factorial in which three concentrations of fluorides were combined with five length groups. Concentrations of fluorides were 250, 325, and 500 p.p.m. The 2-inch length groups ranged from 4 to 14 inches. Since the numbers of fish per length group per concentration varied, a factorial with disproportionate subclass numbers was used (Snedecor, 1946).

LETHAL DOSES OF FLUORIDE

Acute symptomology

Symptoms of acute fluorosis displayed by carp and rainbow trout included apathy and anorexia. The onset of these symptoms was dependent on fluoride concentration and time of exposure. Apathy and anorexia were followed by a period of violent, sporadic movement, loss of equilibrium, and finally death. The near-death symptoms were characterized by the loss of equilibrium and muscle tremors, especially in the dorsal and caudal peduncle regions. Fish died in a state of partial or total muscle contraction. During the period in which these symptoms were displayed, the fish appeared to secrete an excessive amount of mucus, especially in the higher range of fluorides.

Changes in serum proteins also occurred (Table 1). These determinations were made on carp displaying muscle tremors and those displaying no symptoms. Proteins designated as 2, 5, and 7 showed significant differences. The fish which displayed muscle tremors had a reduction in the level of protein 2 and an increase in the levels of proteins 5 and 7. Protein 4 was significant at the 90 percent level of confidence. This result might be interpreted as a change in protein level. The change indicates that it decreases with the onset of tremors.

Rainbow trout embryos, which died within the egg, displayed symptoms similar to those of adult fish. Violent movement often ruptured the vitelline membrane, and the embryo succumbed *in vitro*. If the membrane was not ruptured the embryo died *in vivo*. Death of the embryo was usually concurrent with coagulation of the yolk protein. If the vitelline membrane ruptured, the embryo invariably left the egg sac headfirst. Not infrequently, embryos were caught in the membrane. If these fish lived, they often had deformed spines.

TABLE 1.—*Mean percentage serum protein levels from normal carp and carp displaying symptoms of fluoride intoxication determined by paper electrophoresis analysis*

Symptom	Protein designation								
	1	2	3	4	5	6	7	8	9
Tetany	3.41	4.26	5.57	2.44	36.47	10.06	12.01	9.81	11.11
No tetany	4.94	8.22	5.37	3.24	27.25	10.68	8.71	8.32	11.17
Difference	−1.53	+3.96*	+0.20	−0.80	+8.22*	+0.28	+1.17*	+0.49	−0.06

*Significant at the 95 percent level of confidence.

The length of time it takes eggs to hatch in fluoridated water varies with the concentration. Eggs hatch earlier in high concentrations but are not as advanced in development. An analysis of variance of the hatching time of 400 rainbow trout eggs subjected to 0, 100, 200, and 300 p.p.m. of fluoride indicates that a significant linear regression exists in hatching time against concentration.

Rainbow trout

The toxicity of fluorides to rainbow trout was determined on fish ranging in length from 4 to 8 inches when the water contained less than 3 p.p.m. of calcium or magnesium. At 55° F. the L.C. 50 varied between 2.7 and 4.7 p.p.m. fluoride (95 percent confidence level) in a 480-hour experiment with the last recorded mortality occurring at 218 hours. The sensitivity (the slope of the regression formula) of the fish to concentration of fluorides was between 2.43 and 4.01 probits of response to one unit change in the log concentration of the toxin at the 95 percent level of confidence. This relationship held to the formula

$$Y = 3.19 + 3.27X$$

where Y is the response in probits and X is the log of the fluoride concentration (Figure 1).

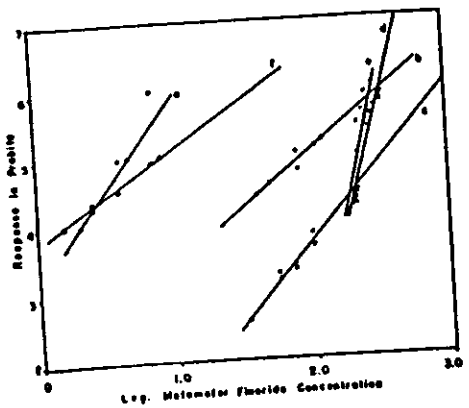

FIGURE 1.—The response of rainbow trout, carp, and rainbow trout eggs to concentrations of fluoride: (a) rainbow trout at 55° F.; (b) carp at 65° F.; (c) rainbow trout eggs at 46° F.; (d) rainbow trout eggs at 55° F.; (e) rainbow trout eggs at 60° F.; (f) rainbow trout from hatching to the absorption of the egg sac at 60° F.

Carp

The toxicity of fluorides to carp was determined on fish ranging in length from 4 to 14 inches. The calcium and magnesium concentrations were below 3 p.p.m. At temperatures ranging between 65° and 75° F., the L.C. 50 was between 75 and 91 p.p.m. fluoride (95 percent confidence level). The sensitivity was between 1.92 and 1.94 probits of response per unit change in the log metameter (95 percent confidence level). The relationship between the probits of response and the log metameter of the fluoride (Figure 1) followed the formula, $Y = 1.93X - 1.31$.

The size of carp influenced the length of time required to produce death at any concentration, but did not determine the L.C. 50 (Table 2). As the size of fish increased, a longer time elapsed before mortality occurred at any concentration.

TABLE 2.—*Mean number of hours before death of carp in varying length groups at different concentrations of fluoride*[1]

Length group (inches)[2]	Concentration (p.p.m.)[2]			Mean
	250	325	500	
4-5	110.67	112.95	35.01	86.21
6-7	121.25	123.53	45.50	96.79
8-9	121.40	126.68	48.71	99.94
10-11	171.34	173.62	95.68	146.88
12-13	149.41	151.69	73.75	124.95
Mean	135.41	137.69	59.75	—

[1]The values are corrected for unequal subclass numbers (Snedecor, 1946).
[2]Significant at the 99 percent level of confidence.

Rainbow trout eggs and fry

Rainbow trout eggs at identical stages of development were tested in water and varying temperatures with calcium and magnesium concentrations of less than 3 p.p.m. At 46° F. the L.C. 50 was between 222 and 273 p.p.m. fluoride (424 hours); at 55° F. it was between 242 and 261 p.p.m. (214 hours); and at 60° F. it was between 237 and 281 p.p.m. (167 hours). All estimates are at the 95 percent confidence level.

The sensitivities were established between 1.88 and 2.16, 7.14 and 7.24, and 10.65 and 10.97 probits of response per unit of the log metameter of the fluoride for 46° F., 55° F., and 60° F., respectively. The relationships between the log metameter of the fluoride and the probits of response for the three temperatures are expressed by the following formulae (Figure 1): 46° F., $Y = 2.02X - 0.17$; 55° F., $Y = 7.19X - 12.36$; and 60° F., $Y = 10.81X - 21.08$.

Another experiment with rainbow trout embryos and the fry extending to the time of yolk-sac absorption was conducted at 60° F. for 825 hours. The L.C. 50 was between 61 and 85.3 p.p.m. fluoride, and the sensitivity was between 1.28 and 1.40 probits of response per unit change in the log metameter (95 percent confidence limit). The relationship between the log metameter and the probits of response (Figure 1) is expressed by the formula, $Y = 1.34X - 2.52$.

FACTORS AFFECTING FLUORIDE TOXICITY

Temperature

Temperature appears to have an effect on both the sensitivity and the L.C. 50. An increase in the temperature resulted in an increase in the sensitivity from 2.02 at 46° F. to 10.81 at 60° F. (Figure 2). This experiment was conducted on rainbow trout eggs and progressed until all the eggs hatched or responded to the toxin. The L.C. 50 also increased from 246 p.p.m. at 46° F. to 251 p.p.m. fluoride at 60° F. (Figure 2). A test of the hypothesis that the variances of the L.C. 50's or the sensitivities are equal (Dixon and Massey, 1951) indicates that significant differences exist, or that the variances at different temperatures were sufficiently different to consider them from separate populations.

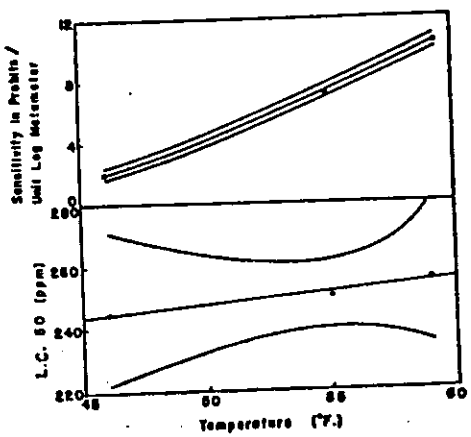

FIGURE 2.—The empirical relationship between sensitivity and temperature (upper) and the L.C. 50 and temperature (lower) of rainbow trout eggs.

Calcium

The relationship between the concentrations of calcium and fluoride ions and the L.C. 50 of rainbow trout subjected to varying combinations of calcium and fluoride was determined by plotting the log of the ratio of fluoride to calcium against the probit of responses to the varying combination of calcium and fluoride. A straight line relationship from which the L.C. 50 can be determined was found (Figure 3). The L.C. 50 was determined between 1.01 and 4.22 [fluoride] / [calcium] at the 95 percent confidence level. The sensitivity of the rainbow trout to the ratio of fluoride to calcium was between 1.71 and 2.35 probits of response per unit change in the log of the ratio. The relationship between the response and the log of the fluoride/calcium ratio (Figure 3) is expressed by the formula,

$$Y = 2.33 - 2.03X$$

where Y is the response in probits and X is the logarithm of the ratio between the fluoride concentration and the calcium ion concentration plus one unit characteristic.

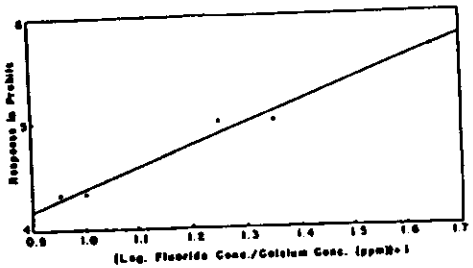

FIGURE 3.—The response of rainbow trout to combinations of fluoride and calcium in the medium (expressed as the ratio between fluoride and calcium).

Chloride

The effect of chloride on the toxicity of fluoride was tested with the mosquitofish, *Gambusia affinis* (Baird and Girard). The toxicity of a given level of fluoride was increased when the total anion normality was increased by the addition of sodium chloride. At 500 p.p.m. fluoride the mortality time for

50 percent of the fish was 14 hours, and 100 ercent was 19 hours. When the total anion normality was increased from 0.026 (fluoride t 500 p.p.m.) to 0.26 by the addition of odium chloride the mortality times were decreased to 30 minutes for 50 percent of the h and 1 hour for 100 percent. The controls ith a total anion normality of 0.26 introuced as sodium chloride produced no apparent ill effects.

FLUORIDE UPTAKE

Uptake in muscle tissue

Variation in the amounts of fluoride in muscle tissues was high in both carp and rainbow trout. This variation was due in part to an inability to extract integumentary bones from the muscle samples. Since the bones held high concentrations of fluorides, any fluctuation in the amount of bone tissue in the sample caused very significant variations in the apparent fluoride content of the muscle tissues. The values obtained for the muscle tissues could, however, be lumped into high and low medium fluoride concentration categories, and the variance produced by the two categories could be demonstrated. Since low levels of fluorides in the water produced a concentration in the muscle tissues of 2.95 p.p.m. and the high levels produced 20.83 p.p.m., it is concluded that the fluoride concentration in the muscle tissue increases with an increase in the fluoride concentration in the medium (99 percent confidence level).

Uptake in osseous tissue

Fluoride uptake in the osseous tissues is a function of time and fluoride concentration in the medium. Analysis of the data indicates that concentration in the medium has the greater effect on the fluoride concentration in the bone. Cancellous bones from the head region of both rainbow trout which lived and died during the experiments were analyzed. When the mean rate of fluoride uptake by bone per hour is plotted against concentration in the medium, a quartic polynomial relationship results (Figure 4A). A similar relationship existed for both the cancellous and skeletal bones of rainbow trout (Table 3).

The bone samples from these fish were aken at the termination of the experiment. This procedure secured samples from fish hat had died from the toxin and from fish that lived to the termination of the experiment. Use of two types of samples could be a source of variation if the rates of uptake from both types were analyzed together. This variation could be eliminated if one or the other types were analyzed separately (Figure 4B).

Since the fluoride in the medium was the only source of fluoride, it could have been an index to the fluoride in the fish bone. On this assumption, the fluoride concentration of the medium becomes the concentration of one of the reactants in the reaction in which fluoride

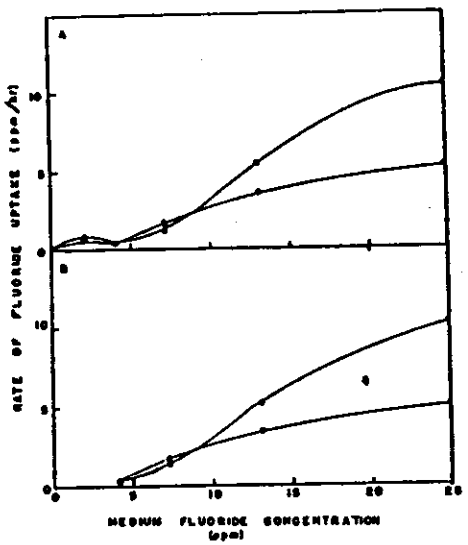

FIGURE 4.—The empirical relationships between the rate of fluoride uptake and the medium concentration of fluoride for cancellous and skeletal bones of rainbow trout (circles represent skeletal bone and dots represent cancellous bone): A. for all fish undergoing experimentation; B. for only fish that succumbed to fluoride intoxication during the experiment.

is deposited in the bone, and the rate of fluoride uptake becomes the velocity of the formation of products in the same reaction. A double reciprocal plot of the type presented by Lineweaver and Burk (1934) resulted in significant (at the 99 percent level of confidence) linear regressions for cancellous and skeletal bones in both the rainbow trout and the carp.

TABLE 3.—*Relationships of the reciprocal of fluoride uptake in various bones to the reciprocal of the fluoride concentration in the medium*

Species	Bone	Formula[1]	Illustration
Rainbow trout	Cancellous	$Y = 0.003 + 1.979X$	Fig. 5a
Rainbow trout	Skeletal	$Y = 0.014 + 1.431X$	Fig. 5b
Carp	Cancellous	$Y = 0.004 + 12.751X$	Fig. 5c
Carp	Skeletal	$Y = 0.023 + 25.520X$	Fig. 5d

[1]Where Y is $1/v$ and X is $1/[S]$.

The relationships are expressed by the formulae tabulated in Table 3 and illustrated in Figure 5. These relationships are characteristic of second-order reactions. Since they occur in a biological system they could be assumed to be enzymatic reactions of the type,

$$[E] + [S] \rightleftharpoons [ES] \rightleftharpoons [P] + [E]$$

where $[E]$ is the enzyme concentration, $[S]$ is the fluoride concentration, $[ES]$ is the enzyme-fluoride complex, and $[P]$ is the product concentration. The formulae of Table 3 would then assume the Lineweaver-Burk form,

$$1/v = (Km/Vmax)\ (1/[S]) + 1/Vmax$$

where v is the velocity of the formation of the products, Km is the Michaelis constant, $Vmax$ is the maximum velocity, and $[S]$ is the fluoride concentration.

Fish from natural populations offered another opportunity to check the fluoride uptake by the bones. Brown trout from the Firehole River in Yellowstone National Park

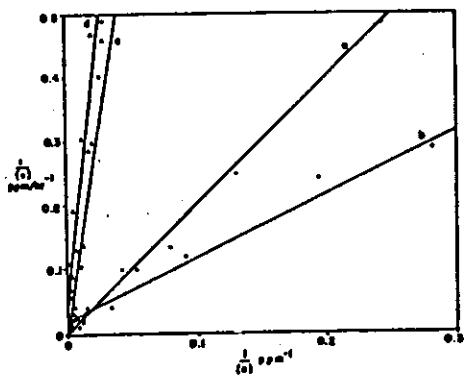

FIGURE 5.—The relationships between the reciprocals of the rates of uptake and the reciprocals of the medium concentration of fluoride for: (a) rainbow trout cancellous bone; (b) rainbow trout skeletal bone; (c) carp cancellous bone; (d) carp skeletal bone.

have a tendency to accumulate fluoride in the bones. This river contains from 1 to 14 p.p.m. fluorides. The fish collected ranged in age from less than 1 to 3 years and from 142 to 400 millimeters in total length.

Regression of the fluoride concentration in the bone on the length of the fish proved to be significantly linear at the 95 percent level of confidence. This relationship (Figure 6) for the cancellous bone is expressed by the formula, $Y = 1.518X + 328$, and the relationship for the skeletal bone by the formula, $Y = 5.501X - 471$, where Y is the concentration of the fluoride in the bone in parts per million and X is the length of the fish in millimeters.

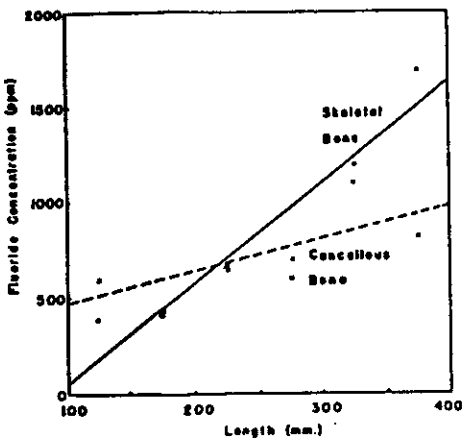

FIGURE 6.—The relationship between the fluoride concentration in cancellous and skeletal bone and length of brown trout from the Firehole River, Yellowstone National Park.

Uptake by rainbow trout eggs

Data on uptake of fluoride by rainbow trout eggs were treated similarly to bone data. Since the analysis for fluoride was on a group of eggs, mortality time was the average time for each concentration. The analysis was on both eggs and freshly hatched fry combined since it was difficult to separate the eggs that died from the toxin and the fry that died shortly after hatching in the toxin.

Significant linearity (99 percent confidence level) was observed in this regression. The relationship (Figure 7) between $1/v$ and $1/[S]$ is expressed by the formula, $Y =$

$25.35X - 0.51$, and is similar to those for uoride uptake in the bones except that the intercept is a negative value.

Histology

Increasing the concentration of fluoride in e medium appears to have an effect on the opulation of mucous cells in the epithelium the gill filaments. In rainbow trout an in-ease of mucous cell density from 0.31 at p.p.m. fluoride to 0.52 at 25 p.p.m. fluoride as observed (Figure 8). The linearity of this lationship was significant at the 99 percent vel of confidence and followed the formula, $= 0.25X + 9.47$, where Y is the count of ucous cells per 30 epithelial cells and X is e concentration of fluorides in the medium parts per million.

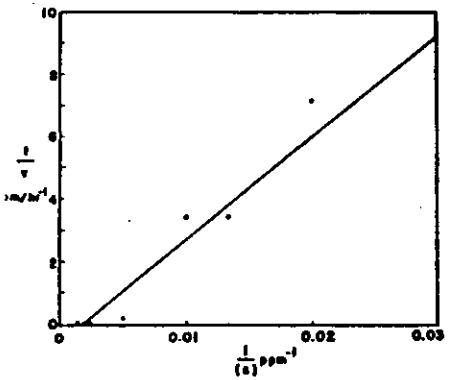

FIGURE 7.—Relationship between the reciprocals f the rate of uptake and the medium fluoride concentration for rainbow trout eggs.

The epithelial tissue in the head region of ainbow trout fry subjected to two ranges of uoride concentrations (0 to 25 p.p.m. and 50 to 335 p.p.m.) also indicated an increase the mucous cells with an increase in the uoride concentration. The tissue upon hich determinations were made was in-gumentary epithelial tissue located dorsally etween the eyes. The population density of e mucous cells ranged from 0.367 in 0 p.p.m. uoride to 0.867 in 335 p.p.m. fluoride (Fig-re 8). The relationship between the mucous ell density and the fluoride concentration of e medium showed significant (99 percent vel of confidence) curvilinearity. Since the

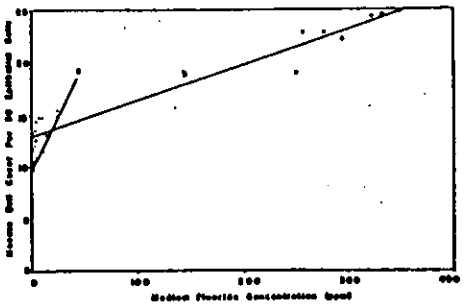

FIGURE 8.—Relationship between the mucous cell count per 30 epithelial cells and the medium fluoride concentration from (a) the gill filament and (b) the inter-ocular integument of rainbow trout.

fluoride concentrations were not at equal increments but rather at two widely spaced portions of the range, no attempt was made to determine the extent of curvilinearity, but there appeared to be a quadratic polynomial relationship.

In addition to changes in the epithelial tissues, changes also appeared to occur in one of the glands. Hypertrophy of the ultimobranchial gland (homologous to the parathyroids in mammals) appeared to occur in fish exposed to higher concentrations of fluorides. This observation is based on comparison with glands of fish maintained as controls (Figure 9).

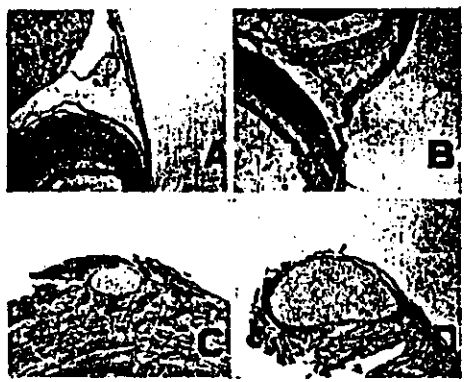

FIGURE 9.—Photomicrographs of A, integumentary epithelium of rainbow trout from 0 p.p.m. fluoride x 150; B, integumentary epithelium of rainbow trout from 250 p.p.m. fluoride x 150; C, ultimobranchial gland of rainbow trout from 0 p.p.m. fluoride x 400; D, ultimobranchial gland of rainbow trout from 4 p.p.m. fluoride x 400.

EFFECTS OF THE TOXIN ON THE FISH

The symptoms of acute toxicity of fluorides to rainbow trout and carp are similar to those described by DeRoos for goldfish. The general lethargy, violent movements, and tetanic-like death were identical in both studies. The lethargy observed for the carp and rainbow trout, however, appeared to be a function of intoxication rather than a function of osmotic pressure differentials as stated by DeRoos. This reaction is ascribed to observations in which both the carp and the rainbow trout reacted identically whether or not they were "tempered" to the osmotic concentrations of the fluoride. The symptoms of lethargy and violent, erratic movement are analagous to some of those displayed by higher vertebrates (Greenwood, 1956).

The embryos of rainbow trout displayed symptoms similar to those of larger fish. This implies that fluoride ions transgress the chorion and are made available to the embryo. Shanklin (1954) demonstrated that fluoride has the effect of reversing the calcium flux across the chorion of *Fundulus* eggs and of building up a concentration of fluoride within the egg.

Ellis *et al.* (1948) indicate that fish eggs subjected to 1.5 p.p.m. fluoride are delayed from 7 to 10 days in hatching beyond comparable eggs in water containing no fluorides. For a higher range of concentration rainbow trout eggs displayed a very significant negative slope for the regression of hatching time on concentration of fluorides. The earlier hatching is probably the result of violent contortions of the embryos intoxicated with fluorides but could be a result of structural disruption of the proteins of the vitelline membrane and the chorion.

Proliferation of mucous cells in the epithelium of fish subjected to fluorides appears to corroborate the subjective observation that there is an increased secretion of mucus by such fish.

The fact that the fluoride concentration in bones increases with concentration in the medium was not surprising since this phenomenon has long been established with higher vertebrates. The experiments performed to establish this in carp and rainbow trout, however, fell somewhat short of direct demonstration. Since the bones taken for analysis were from fish that died as a result of acute intoxication or from fish that lived past the critical acute toxicity point of the experiment, two factors tended to confound each other in the analysis: (1) the period of time each fish was subjected to a given concentration of fluoride and (2) the concentration of fluoride to which each fish was subjected.

The concentration of fluoride in the bones increases with the concentration of fluorides in the medium via a second-order reaction. Since this reaction is in a biological system, it is assumed to be an enzyme-catalyzed reaction. Fluoride, in order to be placed in a position to be absorbed by the bone under the simplest possible conditions, must pass through several physiological phases. It must first transgress the gill or integumentary epithelium into the blood stream which carries it to the bone where it must transgress the capillary walls into the osteo interstices.

Similar observations were made on the rainbow trout eggs (Figure 7). This work was confined to an analysis of fluoride content of the entire egg rather than any specific part. The fluoride must transgress the chorion in an activated fashion, *i.e.*, it must be actively transported across the membrane, presumably by an enzymic reaction. Shanklin (1954) made similar observations on the uptake of fluoride by *Fundulus* eggs.

Another point that requires discussion is the difference of fluoride uptake by the skeletal and cancellous bones. The reciprocal of the velocity of fluoride uptake by the bones changes 1.979 units per unit change in the reciprocal of the fluoride concentration in the medium in the cancellous bones and 1.431 in skeletal bones of rainbow trout. This difference in the slopes is believed to be of small significance. The bones of carp displayed an almost two-fold difference between the slopes of their relationships. The cancellous bones had a slope of 12.7 and the skeletal bones 25.5. This difference is probably related to differences in the amount of non-osseous material which remained on the bones after they were cleaned.

Fluorides tend to occur in fish blood in unassociated, ionic form and associated with another ion or molecule. Because of its high electro-negativity the fluoride ion is more likely to be associated with the electro-positive elements such as calcium and magnesium ions. Gurd (1954) indicated sodium fluoride changes the isoionic point of human serum

albumin to an extent more than twice the range produced by sodium chloride. This indicates fluoride is bound to the protein. A change in the isoionic point of the protein would result in a changed electrophoretic pattern. This appeared to happen to several of the proteins in the carp serum we tested (Table 1).

The determination of acute lethal concentrations of fluoride is dependent on an accurate description of symptoms. The symptoms presented here are sufficiently accurate to separate the fish that die from acute intoxication and from other causes. The time to produce acute symptoms varies with the concentration. An evaluation of the lethal concentrations or the sensitivity cannot be reached, therefore, until all fish exhibiting the symptoms defined for acute intoxication have succumbed. For this reason a specific time interval for the experimentation was not incorporated into the design. The length of the experiment is dependent on the time required for the last of the fish in the group to succumb to the effects of acute intoxication. Thus, in our definition of acute intoxication, a subjective interpretation of the point when the last of the fish dies must be made.

The lethal doses and the sensitivities presented here are valid only for the conditions under which the experiments were conducted. A number of variables were shown to have effects on either the sensitivity, the L.C. 50, or the length of the experiment.

The size of the fish has a very definite relation to the length of the experiment as reflected by the time required for the fish to succumb to the toxin (Table 2). This, however, has no apparent effect on the L.C. 50 or the sensitivity in the probit analysis.

Temperature has a definite effect on the sensitivity as well as the length of mortality time for rainbow trout eggs (Figure 2). Both of these observations may be attributable to differences in metabolic rates of small and large fish of the same species. The same is true for temperature differences with the higher temperatures tending to increase the rate of metabolism of fish eggs (Brown, 1957). Our findings indicate that the sensitivity of the eggs to the toxin increased with rise in temperature. This indicates that the mortality due to fluoride intoxication occurred more completely in a given concentration, or that all the mortality that resulted from fluoride intoxication occurred within a narrower range of fluoride concentrations. The L.C. 50, on the other hand, increased slightly, but significantly, as temperature increased. This finding appears somewhat paradoxical and requires further investigation.

The calcium concentration of the medium seems to reduce the effectiveness of the toxin (Figure 3). The high affinity fluoride has for calcium tends to reduce the effective calcium concentration in the body especially since some of the biochemical reactions require calcium as an activator. The added calcium in the medium provides a source for replenishment of the calcium required to maintain body functions. The chloride concentration of the medium appears to make the fluoride more toxic when the two are included together in the medium.

DEFENSE MECHANISMS

The defense mechanism against fluoride intoxication is essentially fluoride elimination, which can be accomplished by excretion either through the kidney or the respiratory epithelium. Fluoride may produce many of its toxic effects because it inhibits enzymes which require calcium and magnesium for their activity (Whittaker[2]). The elimination of fluoride, therefore, is instrumental in the alleviation of enzymic inhibition, and can be accomplished by incorporation of the fluoride into the bone as a stable mineral complex (Shupe *et al.*, 1955).

DeRoos discusses the similarities between epithelial cells of fish subjected to fluorides and fish subjected to chlorides. Bevelander (1935) believes that the chloride-secreting cells may be modified mucous cells and that chloride may be secreted (associated with the mucin). The increase of the density of the mucous cells in both the gills and the integumentary epithelium of the head region in rainbow trout (Figures 8 and 9) is similar to that noted in goldfish (DeRoos). The mucous cells in rainbow trout and goldfish subjected to fluorides appear to have morphological similarities to the chloride-secreting cells in *Fundulus* and may function as fluoride-secreting cells.

[2]Whittaker, V. P. (1954) Fluorides as enzyme inhibitors. Symposium on fluorides. Univ. of Cincinnati, May 11, 1954. (Mimeo.)

The apparent hypertrophy of the ultimobranchial gland in the rainbow trout that were subjected to fluorides and the display of tetany just prior to mortality are relatively good indications of a calcium deficiency. Turner (1955) indicated tetany can be the result of calcium deficiency in body fluids and that hypertrophy of the parathyroid (ultimobranchial in fish) is often a result. Fluoride tends to form stable complexes with dissolved calcium in the blood and thereby reduces the ionic calcium concentration. It will also react with calcium serving as activators of certain enzyme systems. Fluoride apparently replaces the hydroxyl and bicarbonate groups in the surface of the mineral face of the bones (Neuman *et al.*, 1950) and consequently forms a very stable mineral complex with calcium. Thus, calcium becomes less available for mobilization from the bone. All these factors apparently combine to effect the hypertrophy of the ultimobranchial body in fish. It is significant that calcium in the medium has the effect of delaying the symptoms and apparently acts as a reservoir for body calcium.

The formation of a stable mineral complex with fluoride in the bone would act as a means of eliminating fluoride as well as calcium from systemic circulation, and it was noted that the uptake increases with an increase in the substrate concentration (Figure 5). This response is characteristic of a second-order reaction. If the mucous cells in the epithelium are assumed to act as a fluoride-secreting mechanism, and if their proliferation is assumed to be a function of the fluoride concentration in the blood, the tendency would be toward an increased concentration of blood fluorides with increased concentration of medium fluorides. The effect on the velocity of the fluoride uptake on the bones would then be similar to the relationships presented here.

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SHORT PAPERS AND NOTES

The Appearance of Pink Salmon, *Oncorhynchus gorbuscha* (Walbaum), in Lake Superior

Two salmonid fishes were caught in the fall of 1959 by two anglers in or just above the mouths of two Minnesota streams flowing into Lake Superior and were turned over to the Minnesota Department of Conservation. They were identified as pink salmon, *Oncorhynchus gorbuscha* (Walbaum), and have been deposited in the collections of the University of Minnesota. One fish was caught by Willard J. Negard, September 11, 1959, in the mouth of the Cross River in Cook County and the other by William A. Vancalberg, September 19, 1959, in the Sucker River just below Highway 61 bridge in St. Louis County. These streams support substantial runs of both brown trout *(Salmo trutta)* and rainbow trout *(Salmo gairdneri)* from Lake Superior.

One of the fishes had been eviscerated before it was turned over to the Department of Conservation and measured 19 inches total length. The other fish was intact and measured 19½ inches total length. Both were males and the non-eviscerated specimen had well developed testes. Both were distinctly humped anterior to the dorsal fin and had slightly hooked upper jaws indicating that they had started to develop the secondary sexual characters which Davidson (1935) states appear in the last 35 to 45 days of their life cycle. Both of the fish had the characteristic markings of the pink salmon. They were well marked dorsally with relatively large dark spots and had the characteristic large rectangular spots on the upper part of the deeply forked caudal fin. One had 212 scale rows and the other had 210 scale rows passing through the lateral line. The identification was verified by Dr. Frederick A. Davidson, formerly of the U. S. Fish and Wildlife Service, who saw the fish soon after they were deposited at the University.

The capture of two mature pink salmon at different places in the Lake Superior drainage adds to the number of new fishes which have recently appeared in Lake Superior (Eddy and Underhill, 1959) and raises an interesting problem concerning their origin and probability of survival of a population. The completion of the life cycle of the pink salmon in fresh water is unusual. It has been termed an "obligatory anadromous species" by Rounsefell (1958) which he defines as those species which "either cannot reproduce, or cannot do so with sufficient success to maintain a natural population, without residing for some time in a marine environment." Rounsefell also pointed out that no natural fresh-water populations of pink salmon are known to exist, and that it exhibits the highest degree of anadromy of various species of the North American salmonids. Under normal conditions the pink salmon is known to spawn primarily in rivers, with the resulting fry entering the sea soon after development to the free-swimming stage.

Inquiries concerning the possible origin of these salmon were made to the Province of Ontario, the several states bordering Lake Superior, and the U. S. Fish and Wildlife Service. Only the Ontario Department of Lands and Forests gave any information concerning the possible origin of these fish. They stated that in 1955-1956 eyed eggs of pink salmon were hatched in the Port Arthur Hatchery located on Thunder Bay in Ontario about 40 miles north of the Minnesota border. These eggs were collected from the Skeena River in British Columbia during the fall of 1955.

About 518,000 of the resulting fry were subsequently introduced into Goose Creek, a tributary of Hudson Bay, and 221,000 fry were raised to fingerlings for introduction into the Hudson Bay drainage. Provincial authorities suggested that some small accidental escapement into Lake Superior may have occurred from the hatchery during rearing. It was further learned from Richard Ryder, of the Ontario Department of Lands and Forests, that in the process of transferring the fingerling salmon from the Port Arthur Hatchery to a seaplane, there was a known escapement of probably less than one hundred fingerlings into Thunder Bay of Lake Superior.

The Toxicity of Fluoride to Rainbow Trout

by D. W. M. Herbert and D. S. Shurben
Water Pollution Research Laboratory, Stevenage

Introduction

IN districts where fluoridation of domestic water supplies is practised, the amount added to the water is usually that required to bring the concentration to 1 p.p.m. F. The effluent from sewage works serving such districts might thus contain up to this concentration of fluoride; it has been asked whether this is likely to harm fish in rivers to which such effluents are discharged.

There appear to have been no extensive studies of the survival of fish in fluoride solutions except for the work of Neuhold and Sigler (1), who found that the eggs of both carp (*Cyprinus carpio* L.) and rainbow trout (*Salmo gairdnerii* Richardson) were distinctly more resistant than the grown fish. In a very soft dilution water (calcium 0·5 to 4·5 p.p.m., magnesium 0·0 to 0·3 p.p.m.) carp suffered a 50 per cent mortality within 20 days, exposure to 75—91 p.p.m. fluoride, and it can be calculated from the probit regression line fitted to their data that 1·0 p.p.m. F would probably not kill more than 0·1 per cent of a population of similar fish. This mortality is so small that 1·0 p.p.m. fluoride could be considered to be virtually non-toxic to this species. Rainbow trout were more susceptible; in similar experiments of 20 days' duration, a 50 per cent kill occurred in 3·6 p.p.m. F, and it appeared from their data that in very soft waters about 3 or 4 per cent of a population of such fish might be killed within 3 weeks in a concentration of 1·0 p.p.m. F.

Because the 1·0 p.p.m. fluoride which might be present in a sewage effluent would usually be diluted by river water, Neuhold and Sigler's results suggest that concentrations toxic to fish will not normally be present in rivers as a result of the fluoridation of water supplies. However, the results of toxicity trials performed by different authors sometimes show considerable variation as a result of differences in experimental technique. In Neuhold and Sigler's experiments, the trout were kept in the same solutions of fluoride in aerated aquaria, throughout the 20-day experimental period. Preliminary tests showed that the fish did not produce detectable changes in the concentration of dissolved oxygen or ammonia, and no differences could be discerned in the fluoride concentration of the test solutions between the beginning and the end of each experiment. Nevertheless, their technique is open to the objection that other waste products from the fish might have accumulated in the water and modified the toxicity of fluoride. For this reason it was considered that the toxicity of fluoride to trout should be measured by techniques which did not allow metabolic waste products to accumulate.

Experimental Methods

At each concentration of fluoride, 10 yearling rainbow trout averaging 10 cm. in length were immersed in 40-litre quantities of solution which were continuously aerated to keep the dissolved oxygen concentration close to the air-saturation value, and the fish were transferred each day to freshly prepared solutions 15 to 30 minutes after being fed with a proprietary trout food. The most detailed tests were made with a very soft dilution water (hardness 12 p.p.m. as $CaCO_3$) brought from Lake Trawsfynydd in Wales. Additional toxicity trials in hard water (320 p.p.m. as $CaCO_3$) were made with the borehole water supplied to this Laboratory, and also in water of 45 p.p.m. as $CaCO_3$ obtained by mixing Lake Trawsf

Fig. 1. Effect of concentration of fluoride on the survival of rainbow trout in soft dilution water.
Numbers by each line are concentrations of fluoride as p.p.m. F.
Hardness, 12 p.p.m. as $CaCO_3$.
Temperature 14·30 C.

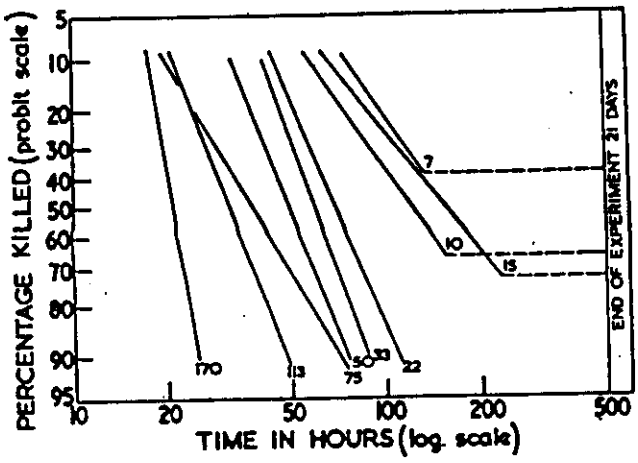
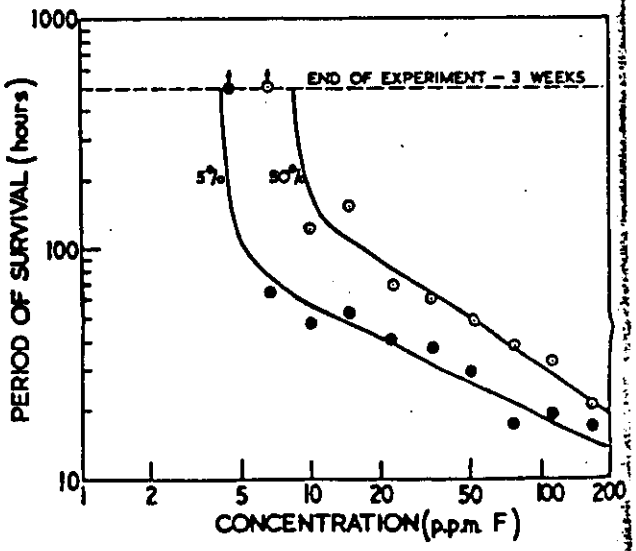


Fig. 2. Relation between fluoride concentrations and time taken to kill 5 and 50 per cent of a rainbow trout population in soft dilution water.



Cynydd and borehole waters. All fish used in the tests were acclimatised to the dilution water and other test conditions for 6 days before the start of the toxicity trials which were continued for a maximum period of 21 days with each dilution water.

Results

Soft water (hardness 12 p.p.m. as $CaCO_3$)

The content of calcium plus magnesium in the soft water used was about 4·8 p.p.m., which is very similar to that of the water used by Neuhold and Sigler. All the fluoride added apparently remained in solution. In the concentrations of fluoride in which all the fish died, the distribution of individual periods of survival was approximately logarithmic normal, and so the times corresponding to any required percentage kill could be interpolated from a line fitted to the plot of probit percentage kill against log time. At the lower concentrations tested these curves showed biological truncation, that is some of the more resistant fish survived until the end of the experiment although their earlier death would have been expected from the trend of the lines fitted to the points for the fish which were killed. The point of truncation occurred at lower percentages as the concentration was reduced (Fig. 1) and this suggests the existence of thresholds of toxic concentration which occur at higher fluoride concentrations for the more resistant fish than for the more sensitive. Additional evidence for the existence of such threshold concentrations is obtained by plotting the logarithm of period of survival against the logarithm of concentration (Fig. 2). As the fluoride concentration is reduced, survival time at first increases slowly, the relation between the logarithms of these variables being a straight line, but with further reduction in concentration the curves turn sharply upwards to become substantially parallel to the axis of log time, so that a small reduction in concentration corresponds to a very great increase in survival time. Neuhold and Sigler's work also suggests that there is a threshold of toxic concentration for fluoride, because in their 480 h experiments the last recorded mortality occurred at 218 h. The concentration of fluoride which kills no more than a given percentage of trout within 21 days will thus be a close approximation to the threshold concentration of fluoride for that percentage of the trout population, and estimates of these threshold concentrations can be read from the probit regression line in Fig. 3. The threshold for 50 per cent mortality is 8·5 p.p.m. F; for 5 per cent it is 4·0 p.p.m. F. The curves in Fig. 2 have been drawn to pass through these concentrations at 21 days.

Harder waters

In waters of hardness 45 and 320 p.p.m. as $CaCO_3$ the log time/log concentration curves for fluoride also indicated the existence of threshold concentrations closely approximating those just killing at 21 days. The results of these tests are summarised in Table 1.

Table 1. Percentage of trout killed within 21 days in sodium fluoride solutions in water of two hardness levels.

Hardness (p.p.m. as $CaCO_3$)	*Fluoride added (p.p.m. F)*	*Percentage killed within 21 days*
45	253	100
	169	100
	113	100
	75	0
	50	0
	0	0
320	250	100
	200	100
	150	90
	100	0
	0	0

In the hardest water a definite precipitate was formed after the sodium fluoride was added, and it usually remained in suspension for several hours before it settled. In the water of intermediate hardness (45 p.p.m. as $CaCO_3$) a persistent opalescence was observed after the fluoride was added. Some attempts to determine the concentration of dissolved fluoride to which the fish were subjected were made by filtering samples from aquaria and determining fluoride by distillation from perchloric acid followed by titration with thorium nitrate. The analytical values for total fluoride (filtrate plus precipitate) were nearly all within 5 per cent of the nominal amount added to the aquaria, but the amount in solution varied greatly from day to day. For this reason the survival of the trout could not be accurately related to the concentration of fluoride in solution, but all determinations of dissolved fluoride in the aquaria immediately above and below the thresholds gave concentrations which were higher than that for the threshold in the softest water from Lake Trawsfynydd: it may, therefore, be concluded that dissolved fluoride was less toxic in the two harder waters than in the softest.

Discussion and Conclusions

It has been suggested that the maximum rate for the continuous discharge of a poison to a river in which a fishery is to be preserved might reasonably be taken as that which, when diluted only by the dry-weather flow of the river, would kill no more than 5 per cent of a population of a sensitive species such as the rainbow trout within a period of 3 months[2]. Although about 4 p.p.m. fluoride produces 5 per cent mortality within 3 weeks, the shape of the curves in Fig. 2 suggests that mortalities would not be much higher even after 3 months at this concentration. The highest concentration of fluoride likely to be present in an effluent from a sewage works is only 1 p.p.m. F, and extrapolation of the probit/log concentration curve in Fig. 3 suggests that this concentration is unlikely to kill more than about 0·01 per cent of a population of trout similar in resistance to those used in the present work. Such a low mortality would be negligible by comparison with the natural death rate to which trout populations are subject. Thus, even in areas where the water is very soft, the highest fluoride concentrations which would be found in undiluted sewage effluents are unlikely to harm a trout fishery. In harder waters (in which fluoride is less toxic), and also in streams where sewage effluents receive dilution with several times their own volume of river water, there would be virtually no risk at all to such fisheries from adding fluoride to the public water supply.

Acknowledgments

This paper is published by permission of the Department of Scientific and Industrial Research.

We are grateful to the staff of the Department's Laboratory of the Government Chemist, who performed the fluoride determinations.

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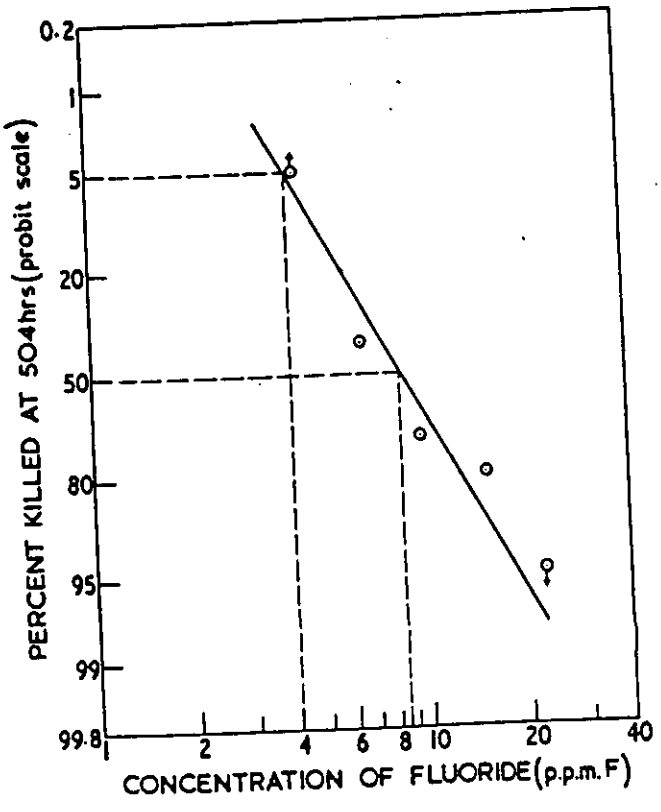
1. Neuhold, J.M., and Sigler, W. F. Effects of sodium fluoride on carp and rainbow trout. Trans. Amer. Fish. Soc., 1960, 89, 358.
2. Herbert, D. W. M. Freshwater fisheries and pollution control. Proc. Soc. Wat. Treatm. and Exam., 1961, 10, 135.

Fig. 3. Probit kill/concentration curve for estimating the threshold concentrations of fluoride in soft water for various percentage of a rainbow trout population.



To: R. Murphy
Ross & Hardies
150 N. Michigan Avenue
Chicago, Ill., 60601

July 17, 1992

Subject: Fluoride Limit Analysis for
Adjusted Rule Change.

Attached you will find two analysis of the 1991 "Outfall 002" fluoride levels. The letter is from our Senior Quality / Reliability Engineer along with the typical Statistical Process Control (SPC) Analysis. The other package is a Lotus analysis that I worked up and yields the same results. I will tell you the logic in each analysis and we can discuss which way to go. I can write up a cover letter and sign it as we did before.

The typical SPC analysis that is used on process control analysis is not exactly proper, but does yield good results. It is used to analyze processes that we have control of, not processes controlled by an independent variable. The rules of the game give requirements for "long term" and "short term" capabilities. Short term capabilities should be 75% of the range and long term tests are allowed 100% of the range. Since we have varied year to year she concludes that 1991 is a "short term" analysis which should theoretically cover 75%. Since the 1991 analysis gave a 7.25Mg/L high the properly toleranced upper limit would be 10.0Mg/L. The 7.25Mg/L is a good reading and not sample error since the other reading taken that day at the other end of the 12MG Lagoon was also high at 6.7Mg/L.

Jackie's SPC analysis indicates that in 1990 the limit of 5.00Mg/L was appropriate. The 1991 readings indicate a definite change in the process which dictates a higher upper control limit.

The other charts give the results of a Lotus standard deviation analysis of the data by year. I set up the table with upper and lower control limits. I included all data whereas a statistical program would eliminate the highest assuming we had control of the process. I charted the results which indicate that the 5.0Mg/L limit was appropriate in 1990 but not in 1991. A straight upper control limit setting for 1991 would be 7.437Mg/L. This would assume that it covers the worst possible operating conditions. I then adjust the numbers for volume and recycle rates.

EXHIBIT

E

The 1991 volumes averaged 780 tons per day ship. We did have periods of time when we ran up to 900 tons per day. Our capacity is 1050 tons per day. The more iron we melt the more limestone we use and the more water we evaporate. Also during 1991 we typically recycled 80% but must increase this to about 90% to meet our NPDES limits. This will be done when we complete our modernization of the water treatment system this September. Both these factors will increase the fluoride levels in our discharge.

From the analysis of the past changes in fluoride levels it would be logical to conclude that the relationship is approximately linear. In reality it is not quite this simple but it works out about right. We do need some allowance for volume changes and recycle and this is a logical approach that I believe we and the Agency can support.

The adjustments I made come up with a fluoride level of 9.76Mg/L or approximately 10.0Mg/l.

Please review the attached package and we can discuss how to organize the analysis. I will be in the plant Thursday and probably Friday July 23 & 24. I will be in and out during the following week. Leave a message on my recorder or call me at home at 217-267-7790.

I'll forward a copy to L. Tucker and K. West but they are out during this two week period.

James F. Schifo, P.E.
Environmental Coordinator

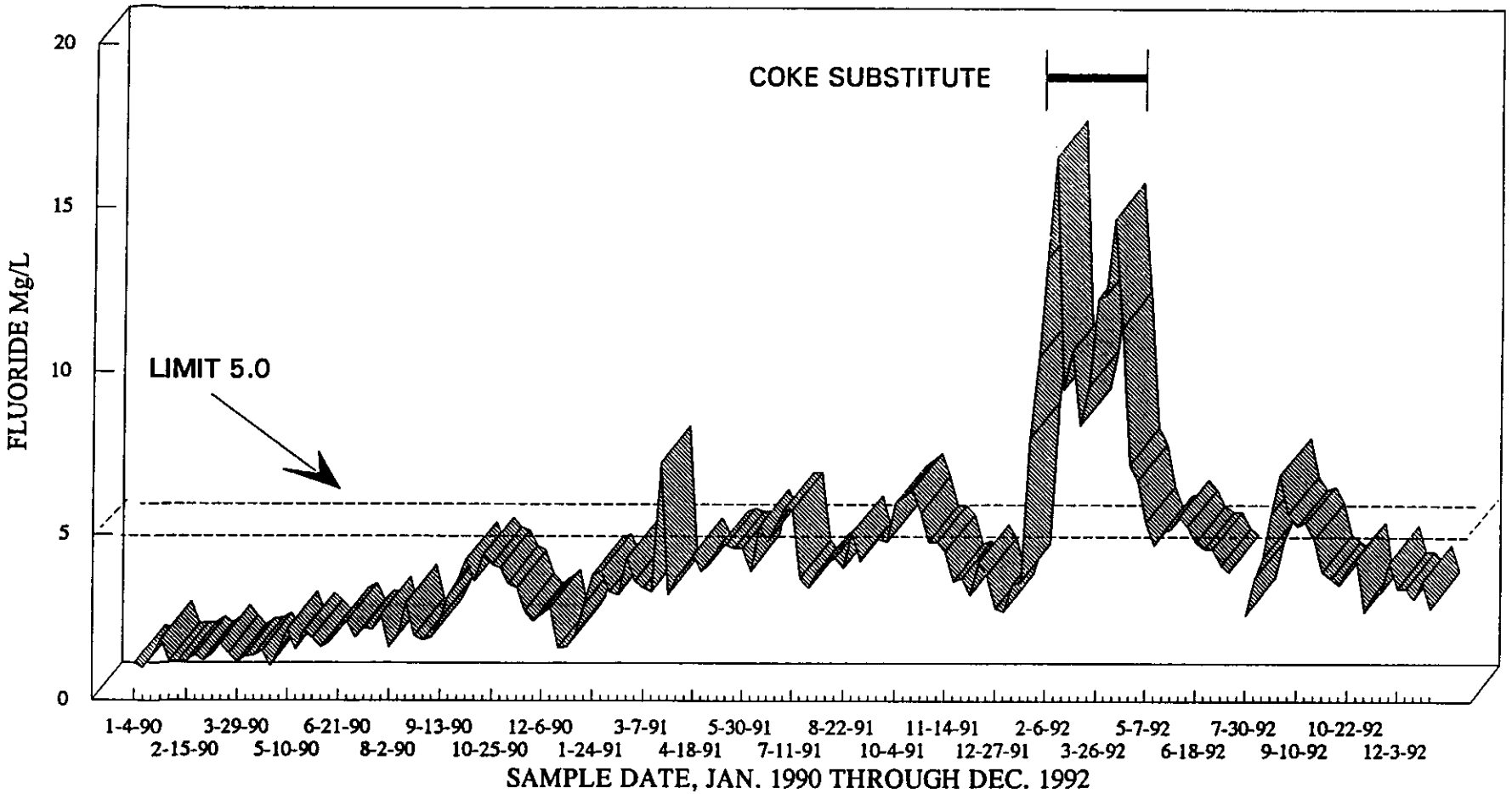
cc: L. Tucker
K. West

FLUORIDE VS. TIME

FEBRUARY 3, 1993

OUTFALL 002

FLTIME



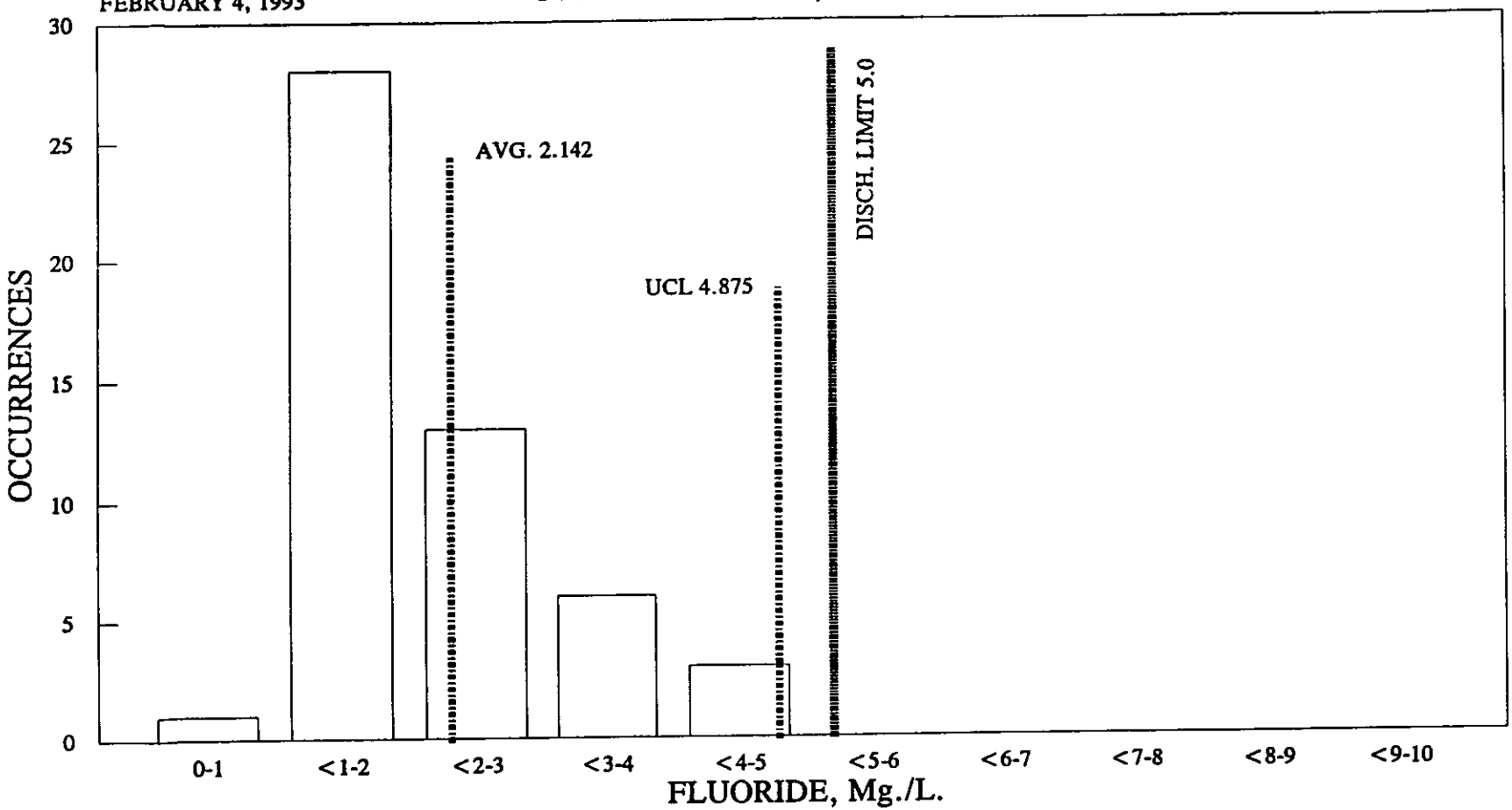
DISCHARGE LIMIT 5.0 Mg/L

1990 FLUORIDE HISTOGRAM

DANVILLE PLANT, OUTFALL "002"

FEBRUARY 4, 1993

JFSFLCT90



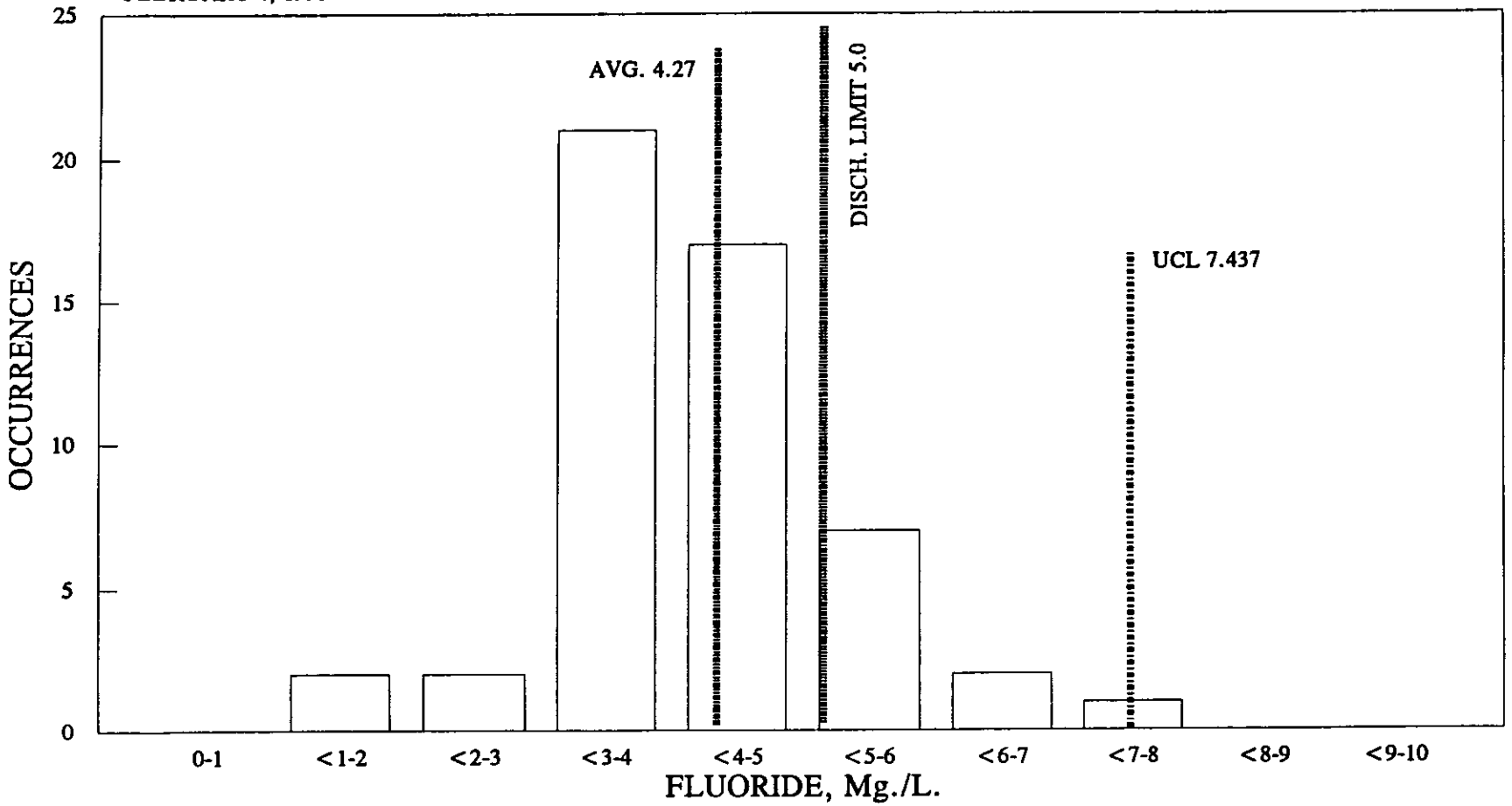
AVG. PROD. 828 TONS/DAY (79%)
MAX. MONTHS 85.7%

1991 FLUORIDE HISTOGRAM

DANVILLE PLANT, OUTFALL "002"

FEBRUARY 4, 1993

JFSFLCT91



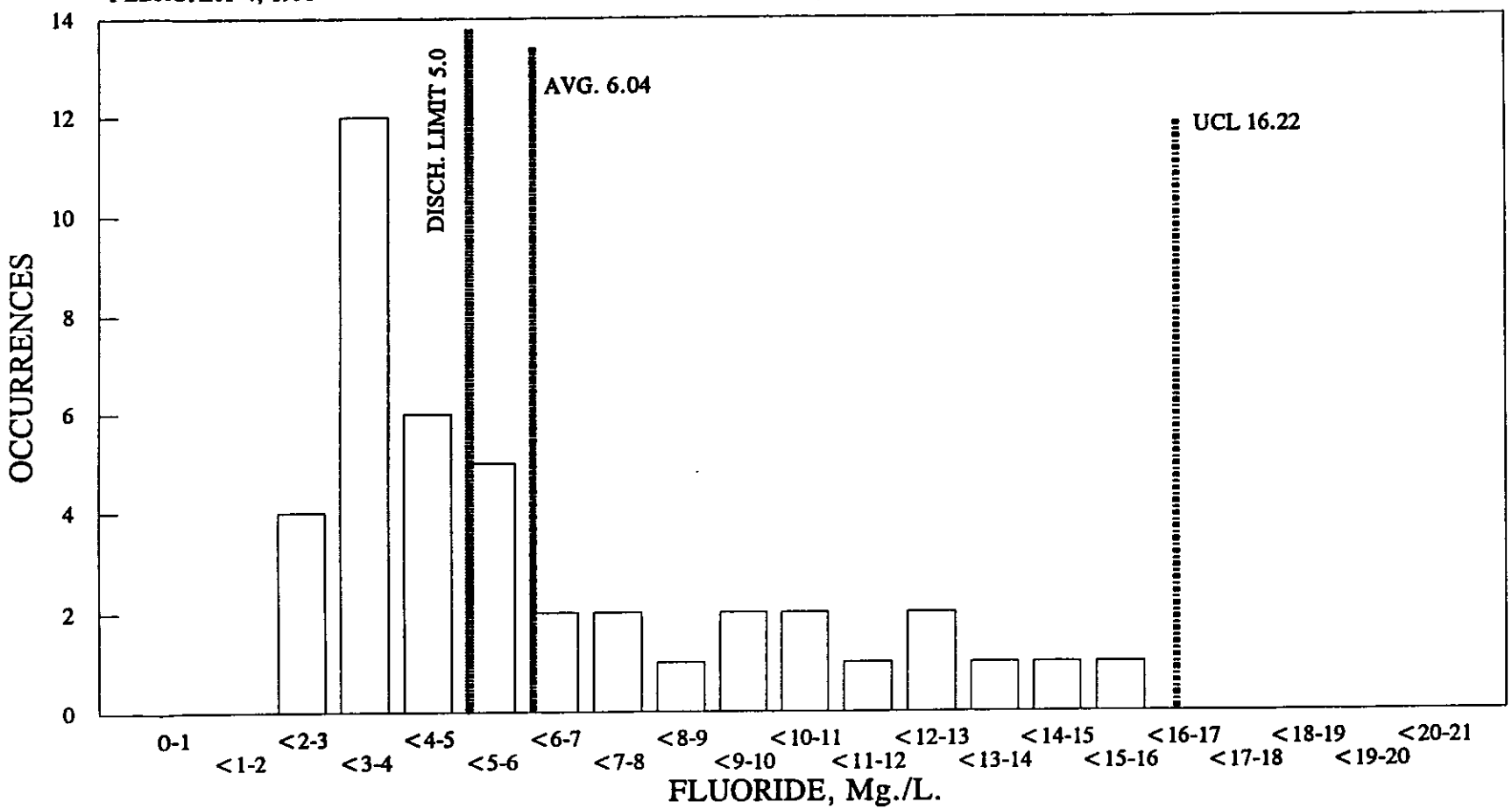
AVG. PROD. 743 TONS/DAY (71%)
MAX. MONTHS 85.7%

1992 FLUORIDE HISTOGRAM

FEBRUARY 4, 1993

DANVILLE PLANT, OUTFALL "002"

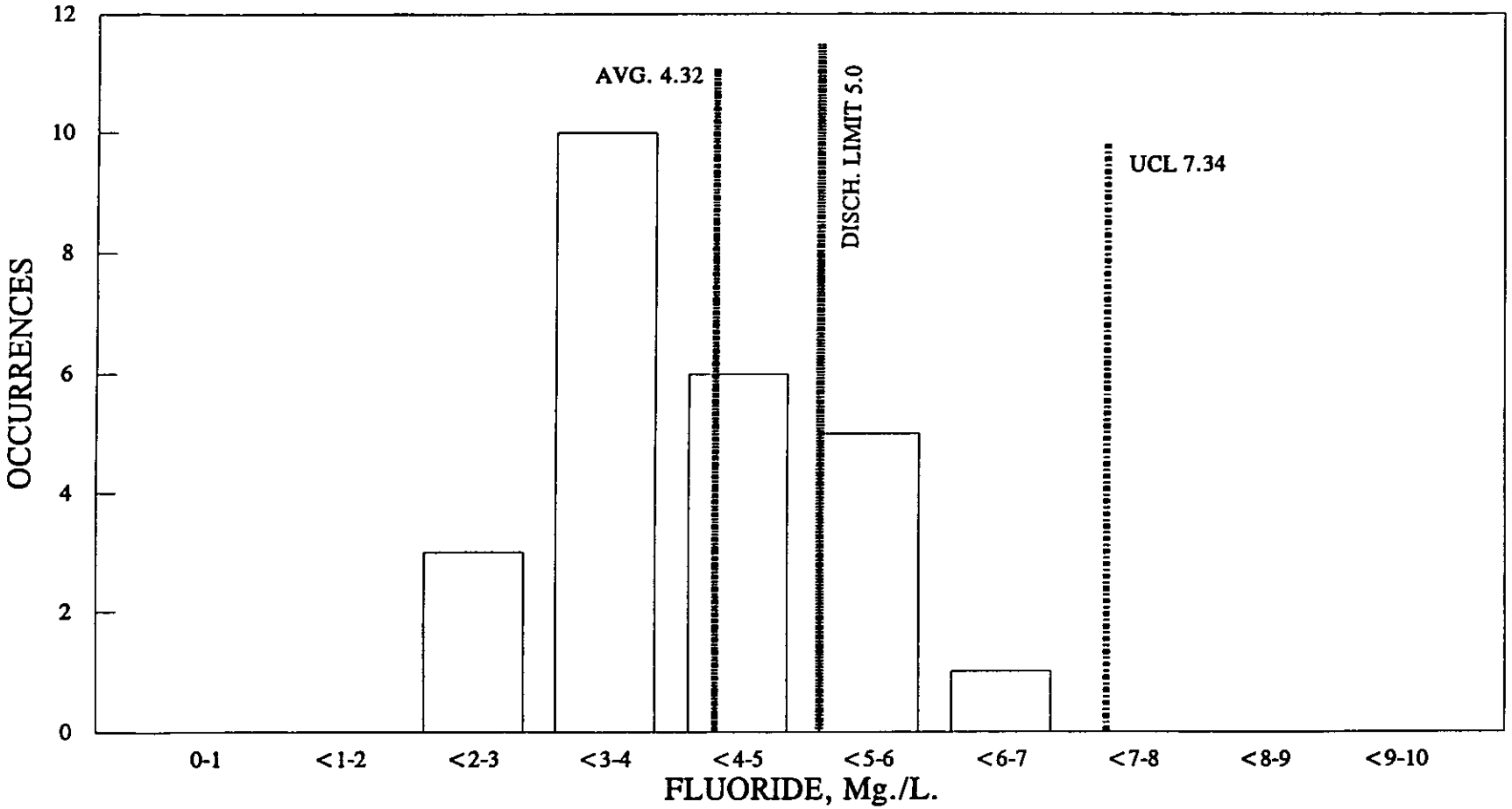
JFSFLCT92



AVG. PROD. 823 TONS/DAY(78%)
MAX. MONTHS 85.7%

1992 FLUORIDE HISTOGRAM, MAY--DEC.

FEBRUARY 4, 1993 DANVILLE PLANT, OUTFALL "002" JFSFLCT92



AVG. PROD. 823 TONS/DAY(78%)
MAX. MONTHS 85.7%

STATISTICAL ANALYSIS OUTFALL "002" FLUORIDE
DANVILLE PLANT, GM POWERTRAIN

02/04/93
JFSFLUOR

(Mg./L)

YEAR	STD. DEV.	AVG	UPPER CONTROL LIMIT UCL	LOWER CONTROL LIMIT LCL
1990	0.911	2.142	4.875	-0.591
1991	1.056	4.269	7.437	1.101

REQUESTED FLUORIDE LIMIT

	SHIP TONS/DAY
1991 PEAK VOLUMES	900
MAXIMUM PRODUCTION LEVELS	1050
1991 RECYCLE RATES	80%
REQUIRED RECYCLE RATES TO MEET NPDES LIMITS. (FALL 1992)	90%

REQUIRED LIMITS = (FLUORIDE CONC.) (1991 UCL) X (MAX. PROD. LEVELS/ 1991 PEAK VOL.) X (REQUIRED RECYCLE/1991 PEAK RECYCLE) =

= (7.437) X (1050/900) X (.90/.80) = 9.76 Mg/L OR **10.00 Mg/L**

FLUORIDE HISTORICAL DATA
DANVILLE PLANT, GM POWERTRAIN

02/03/93
JFSFLUOR

Sample Date	002 Fluoride	TDS	PF Fluoride	RIVER FLUORIDE
1-4-90	1.13	621	1.43	
1-11-90	0.99	572	0.96	0.29
1-18-90	1.31	489	1.22	0.44
1-25-90	1.85	527	1.42	0.30
2-1-90	1.19	577	1.66	0.28
2-8-90	1.20	567	1.62	0.23
2-15-90	1.20	518	1.21	0.22
2-22-90	1.38	520	1.70	0.22
3-1-90	1.21	645	1.47	0.21
3-8-90	1.40	761	1.64	0.22
3-15-90	1.73	617	2.50	0.22
3-22-90	1.42	629	1.39	0.22
3-29-90	1.16	579	1.78	0.21
4-5-90	1.33	571	1.58	0.22
4-12-90	1.36	679	1.98	0.20
4-19-90	1.50	593	1.82	0.22
4-27-90	1.05	674		0.25
5-3-90	1.50	603	1.75	0.30
5-10-90	2.15	589	2.20	0.26
5-17-90	1.54	712	2.37	0.28
5-24-90	2.10	572	2.12	0.18
6-1-90	1.88	723	2.20	0.22
6-7-90	1.60	692	1.60	0.28
6-14-90	1.75	704	2.40	0.21
6-21-90	2.26	622	2.62	
6-28-90	2.39	308	2.70	0.19
7-3-90	1.90	508	2.60	0.90
7-12-90	2.18	292	2.68	
7-19-90	2.15	686	2.60	0.28
7-26-90	2.60	950	2.55	0.25
8-2-90	1.61	825	1.58	0.29
8-10-90	1.95	802	2.10	0.28
8-16-90	2.95	950	3.50	0.39
8-23-90	1.95	843	2.00	0.33
8-30-90	1.83	796	2.45	0.32
9-6-90	1.90	682	2.30	0.34
9-13-90	2.45	1074	2.70	0.45
9-20-90	3.20	1163	3.30	0.42
9-27-90	3.62	1107	3.95	0.36
10-4-90	4.25	1348	4.20	0.50
10-11-90	3.60	1159	3.50	0.20
10-19-90	4.40	1288	4.40	0.20
10-25-90	4.10	1026	4.65	
11-1-90	4.00	1522	4.20	0.24
11-8-90	3.53	1743	2.60	0.60
11-19-90	3.40	1248	3.50	1.00
11-21-90	2.70	1050	3.00	0.28
11-29-90	2.40	1040	2.60	0.30
12-6-90	2.60		3.20	0.07
12-13-90	2.80	1058	2.70	0.36
12-20-90	1.60	740	1.60	
1-3-91	1.62	593	1.92	0.91
1-10-91	2.00	938	2.00	0.38
1-17-91	2.90	938	4.30	0.50
1-24-91	3.80	735	3.60	0.30

1-31-91	3.90	907	2.90	0.90
2-7-91	3.30	1036	3.80	0.26
2-14-91	3.20	997	2.80	0.90
2-21-91	4.30	1078	3.80	0.41
2-28-91	3.60	968	3.10	0.16
3-7-91	3.40	1116	3.40	0.22
3-14-91	3.30	336	4.40	0.14
3-21-91	7.25	1029	6.70	0.10
3-28-91	3.20	1028	3.90	0.15
4-4-91	3.72	1024	3.93	0.27
4-11-91	4.00	958	4.36	0.31
4-18-91	4.40	930	4.63	0.32
4-25-91	3.90	848	4.50	0.40
5-2-91	4.10	928	5.00	0.40
5-9-91	4.60	990	4.80	0.16
5-16-91	4.70	932	4.80	0.20
5-23-91	4.60	968	5.00	0.40
5-30-91	4.60	916	4.80	0.40
6-6-91	3.90	980	4.10	0.40
6-13-91	5.30	1070	4.90	0.40
6-20-91	4.80	1040	5.50	0.30
6-27-91	5.30	1000	7.10	0.40
7-03-91	5.80	1120	6.70	0.22
7-11-91	5.80	1030	4.70	0.33
7-19-91	3.70	910	3.60	0.20
7-25-91	3.40			
8-1-91	3.90	812	5.00	0.60
8-8-91	4.10	830	5.90	0.73
8-15-91	4.30	926	5.10	0.42
8-22-91	4.00	1000	4.20	0.67
8-29-91	5.10	1150	4.20	0.68
9-5-91	4.20	1090	4.40	0.75
9-12-91	4.60	1100	5.20	0.60
9-19-91	4.90	1090	5.60	0.71
9-26-91	4.80	1030	6.00	0.80
10-4-91	6.00	1100	6.60	0.75
10-10-91	6.20	1072	6.70	0.34
10-17-91	6.40	1030	6.30	0.57
10-24-91	5.70	980	6.30	0.57
11-4-91	4.80	760	4.30	0.33
11-7-91	4.80	952	5.00	0.36
11-14-91	4.50	972	4.70	0.27
11-21-91	3.60	892	4.70	0.28
11-27-91	3.70	938	4.00	0.27
12-5-91	3.20	876	3.30	0.25
12-12-91	4.20	920	4.10	0.34
12-19-91	3.80	836	3.00	0.32
12-27-91	2.80	698	2.50	0.32
1-2-92	2.70	658	3.60	0.35
1-9-92	3.50	784	3.90	0.34
1-16-92	3.60	820	4.00	0.38
1-23-92	8.00	894	16.30	0.35
1-31-92	10.50	902	4.50	0.33
2-6-92	13.50	920	15.60	0.34
2-13-92	16.60	972	17.00	0.34
2-20-92	9.50	908	12.70	0.26
3-5-92	10.70	880	10.40	0.28
3-12-92	8.40	850	6.70	0.34
3-19-92	9.60	800	14.00	0.25

3-26-92	12.30	890	14.70	0.25	
4-2-92	12.40	920	14.80	0.27	
4-9-92	14.70	948	16.10	0.25	
4-16-92	11.40	850	10.70	0.29	
4-23-92	7.20	814	7.30	0.27	
4-30-92	6.70	796	7.00	0.00	
5-7-92	5.40	846	5.20	0.19	
5-14-92	4.70	788	5.00	0.19	
5-21-92	5.10	776	4.70	0.31	
5-28-92	5.20	958	5.50	0.32	
6-4-92	5.60	1040	5.70	0.35	
6-11-92	5.30	898	5.20	0.33	
6-18-92	4.80	828	6.10	0.35	
6-25-92	4.60	862	4.90	0.37	
7-2-92	4.60	904	4.40	0.43	
7-9-92	4.10	764	5.00	0.25	
7-16-92	3.90	642	4.70	0.29	
7-23-92				0.23 PLANT DOWN	
7-30-92	2.60	466		0.30 PLANT DOWN	
8-6-92	3.70	596	3.90	0.30	
8-13-92	4.30	694	4.60	0.33	
8-20-92	5.70	776	7.10	0.35	
8-27-92	6.90	946	7.50	0.54	
9-3-92	5.70	946	6.30	0.40	
9-10-92	5.30	900	6.30	0.45	
9-17-92	5.40	866	4.90	0.41	
9-23-92	4.90	830	6.10	0.52	
10-1-92	3.90	724	3.80	0.30	
10-8-92	3.70	738	4.80	0.40	
10-15-92	3.50	810	3.80	0.40	
10-22-92	4.00	754	4.80	0.33	
10-29-92	4.30	778	4.40	0.42	
11-5-92	2.70	668	2.50	0.14	
11-12-92	3.20	658	3.00	0.15	
11-19-92	3.30	772	4.70	0.16	
11-25-92	4.20	810	4.90	0.18	
12-3-92	3.40	708	3.50	0.29	
12-10-92	3.40	788	3.60	0.27	
12-17-92	3.10	900	3.20	0.25	
12-23-92	3.60	874	3.30	0.25	
12-29-92	2.80	730	2.20	0.22	

AVERAGE	4.15	852.66	4.55	0.35	8.74%
1990	2.14	791.28	2.40	0.31	10.59%
1991	4.27	948.96	4.55	0.42	6.21%
1992	6.04	816.55	6.70	0.31	9.83%

CERTIFICATE OF SERVICE

I, DAVID L. RIESER, an attorney, depose and state that I caused copies of the foregoing **Motion to Waive Requirement to Submit 200 Signatures and Petition to Amend Site-Specific Regulation** to be served by depositing copies of the same in the U.S. Mail Chute, located at 150 North Michigan Avenue, Chicago, Illinois, before the hour of 5:00 PM this 23rd day of June, 1993, addressed as follows:

Mary Gade, Director
Illinois Environmental
Protection Agency
P.O. Box 19276
2200 Churchill Road
Springfield, Illinois 62794

Bruce Carlson
Office of General Counsel
Illinois Environmental
Protection Agency
P.O. Box 19276
2200 Churchill Road
Springfield, Illinois 62794

Bill Denham
Department of Energy & Natural Resources
325 West Adams
Springfield, Illinois 62704-1892

DAVID L. RIESER