SHORT-TERM TOXICITY OF FLUORIDE ION (F^-) IN SOFT WATER TO RAINBOW TROUT (Salmo gairdneri) AND BROWN TROUT (Salmo trutta fario)

by

J.A. Camargo* and J.V. Tarazona
Madrid, Spain

SUMMARY: Short-term static bioassays were conducted to determine the toxicity of fluoride ions (F^-) in soft water (hardness average value of 22 ppm $CaCO_3$) to Salmo gairdneri Richardson and Salmo trutta fario Linnaeus. Fry of each trout species were exposed to five different concentrations of sodium fluoride (NaF) and a high concentration of sodium chloride (800 ppm NaCl for S. gairdneri and 1000 ppm NaCl for S. trutta fario) for 8 days. No significant effect on the fish was observed with NaCl. Toxic effects caused by NaF were fundamentally due to F^- ions. The LC_{50} at 96, 120, 144, 168 and 192 h were 107.5, 94.4, 85.1, 73.4 and 64.1 ppm F^- for rainbow trout and 164.5, 135.6, 118.5, 105.1 and 97.5 ppm F^- for brown trout, respectively. Fry showed hypoexcitability, darkened backs and a decrease in respiration before their death. S. gaidneri was significantly ($p < 0.05$) more sensitive to F^- than S. trutta fario. A MATC of 27.6 ppm F^- has been determined for rainbow trout.

KEY WORDS: Fluoride ion; Salmo gairdneri; Salmo trutta fario; Short-term toxicity; Soft water.

Introduction

The fluoride concentration in sea waters normally ranges from 1.2 to 1.4 ppm (1) and most fresh waters contain leas than 0.2 ppm F^- (2). However, the fluoride concentration in surface waters is increasing as a result of industrial pollution (3), which may generate an acute or chronic toxicity to biological communities. In this respect, McClurg (4) has indicated that fresh water organisms may be far more sensitive to fluoride pollution than those living in sea waters, because the toxicity of fluoride is decreased by the formation of innocuous complexes with one or more ions of sea water (5).

Toxic effects of fluoride compounds have been described in aquatic invertebrates as Daphnia magna (2,6), Artemia salina (7), Penaeus indicus (4,8) and Hydropsyche spp. (9). It appears that soft water species are more sensitive to fluoride than those in hard or sea water. In fish, the fluoride toxicity may be influenced not only by such common factors as size (10), species (11,12), and physiological state (13,14), but also by the physicochemical characteristics of the water. Thus, the tolerance of fish to fluoride is increased by low temperatures (15,16) and high levels of calcium hardness (17-19). To this effect, Pimentel and Bulkley (20) found that the 96-hour LC_{50} values for Salmo gairdneri increased from 51 to 193 ppm F^- as water hardness rose

* J.A. Camargo and J.V. Tarazona, Department of Animal Health, CIT-INIA, Embajadores 68, 28012-Madrid, Spain.

from 17 to 385 ppm $CaCO_3$ Gasterosteus aculeatus and P hardness due to the precipit MgF_2).

The principal purpose of toxicity of fluoride ions (F^- Salmo trutta fario, common Peninsula, and to study wh these two trout species in ions.

Fry of rainbow trout (fario) were obtained from a as disease free at our CIT-I tation. In the laboratory, Those fry used in the exp fluoride toxicity bioassays, gairdneri and 123.6 ±20.9 mg

Laboratory bioassays w 20 L. of dechlorinated Madr tion and turbulence were photoperiod was utilized, a of a cooling unit with ther sodium fluoride (NaF pro an tration with an approximate

Hardness, alkalinity, chl pH, water temperature, dis the start and at the end described by APHA (21) an tored daily using an Orion were exposed to sodium fluo

Methods for these stati for standardized laboratory fluoride concentration aqua bioassays were performed tested using ten fish per aq

Test organisms were a prior to fluoride bioassays during toxicity bioassays. were removed daily during daily checked by comparin aquarium.

The LC_{50} values at 96, limits and χ^2 values were Wilcoxon (24), using morta in duplicate for each trou was floating upside down an

from 17 to 385 ppm $CaCO_3$. Smith et al. (12) concluded that LC_{50} values Gasterosteus aculeatus and Pimephales promelas varied with the initial water hardness due to the precipitation of calcium and magnesium salts (CaF_2 and MgF_2).

The principal purpose of this study has been to determine the short-term toxicity of fluoride ions (F^-) in soft water to fry of Salmo gairdneri and Salmo trutta fario, common species in cold-water streams from the Iberian Peninsula, and to study whether there are significant differences between these two trout species in relation to their respective sensitivity to fluoride ions.

Materials and Methods

Fry of rainbow trout (Salmo gairdneri) and brown trout (Salmo trutta fario) were obtained from a Spanish ICONA trout hatchery and were certified as disease free at our CIT-INIA laboratories. No fish died during the transportation. In the laboratory, fish were randomly distributed into test aquaria. Those fry used in the experiments were about two months old and, after fluoride toxicity bioassays, weighed (dry weight) 118.9 ±14.9 mg for S. gairdneri and 123.6 ±20.9 mg for S. trutta fario.

Laboratory bioassays were conducted in glass aquaria each containing 20 L. of dechlorinated Madrid tap water (Figure 1). Necessary water oxygenation and turbulence were produced by an air pump per aquarium. Natural photoperiod was utilized, and water temperature was maintained by means of a cooling unit with thermostat. Test fluoride solutions were prepared from sodium fluoride (NaF pro analysi, Merck), geometrically increasing the concentration with an approximate factor of 1.6.

Hardness, alkalinity, chlorine, chloride, sodium, potassium, amonia, nitrite, pH, water temperature, dissolved oxygen, and conductivity were analyzed at the start and at the end of each toxicity bioassay, using analytical methods described by APHA (21) and Rodier (22). Fluoride concentrations were monitored daily using an Orion model 94-09 specific ion electrode. The trout fry were exposed to sodium fluoride solutions for 8 days.

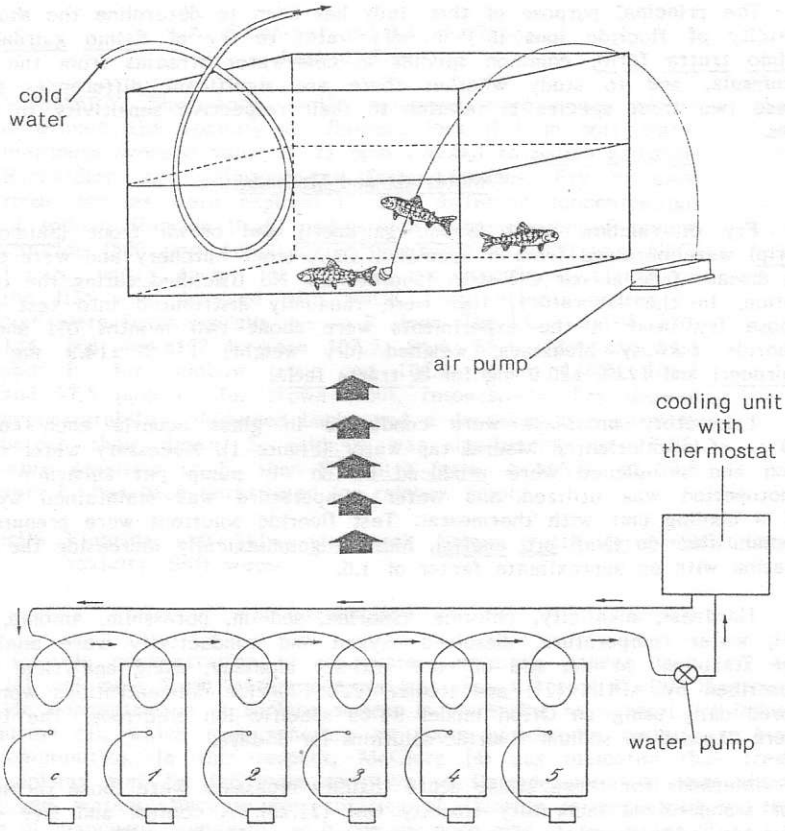
Methods for these static acute toxicity bioassays were those recommended for standardized laboratory toxicity test (21,23). A control and five different fluoride concentration aquaria were used per bioassay (Figure 1). Fluoride bioassays were performed in duplicate. Each trout species was separately tested using ten fish per aquarium.

Test organisms were acclimatized to water quality conditions for 4 days prior to fluoride bioassays and were not fed during the acclimatization nor during toxicity bioassays. No fish died during ithe acclimatization. Dead fish were removed daily during fluoride toxicity bioassays. Sublethal effects were daily checked by comparing fish in fluoride aquaria with those in control aquarium.

The LC_{50} values at 96, 120, 144, 168 and 192 hour, their 95% confidence limits and χ^2 values were determined by the method of Litchfield and Wilcoxon (24), using mortalities and mean assay F^- concentrations obtained in duplicate for each trout species. Death of fish was defined as the fish was floating upside down and not operculating.

Figure 1

Diagram of Experimental Aquaria System Used for Fluoride Toxicity Bioassay. c = control aquarium; 1, 2, 3, 4, and 5 = fluoride aquaria.



The formula of factors (21,24) was applied for obtaining significant ($p < 0.05$) differences between two test trout species. The Maximum Acceptable Toxic Concentration (MATC) was interpolated as the geometric mean of the lowest concentration having a toxic effect and the highest concentration having no toxic effect (25) after 8 days exposure to fluoride solutions.

To find out whether the toxicity of sodium fluoride was due to fluoride ions, sodium and conductivity toxicity tests were conducted parallel to fluoride toxicity bioassays using sodium chloride (NaCl pro analysi, Merck). For this purpose, 10 fry of each test trout species were exposed to a high sodium chloride concentration for 196 hours: 800 ppm NaCl for rainbow trout and

1000 ppm NaCl for brown
Physicochemical parameters
each toxicity test using th
and sublethal effects were c

Mean values of water p
toxicity tests are presented
posure to NaCl. Fry showed
at first, returning to their
lethal effects such as hyp
respiration were not observ
did not differ appreciably
assay.

Mean values of water
bioassay are presented in T
for aquatic organisms (26).
start, and no precipitation
assays.

Mean concentrations of

Mean Values of Water Par
Obtained During NaCl Toxi

Parameter
Water Temperature (°
Alkalinity (ppm CaC
Hardness (ppm CaCO
Dissolved oxygen (pp
Chlorine (ppm Cl_2)
Ammonia (ppm N)
Nitrite (ppm N)
Fluoride (ppm F^-)
Potassium (ppm K^+)
pH
Chloride (ppm)
Sodium (ppm Na^+)
Conductivity (µmhos
Mortality (%)

N.D. = Not Detecte

1000 ppm NaCl for brown trout. These tests were performed in duplicate. Physicochemical parameters were analyzed at the start and at the end of each toxicity test using the same analytical techniques. Possible mortality and sublethal effects were checked every day.

Results

Mean values of water parameters estimated during sodium and conductivity toxicity tests are presented in Table 1. No fish died after 196 hours of exposure to NaCl. Fry showed symptoms of hyperexcitability and hyperventilation at first, returning to their normal state after about 10 hours. However, sublethal effects such as hypoexcitability, darkened backs and a decrease in respiration were not observed during these tests. Values of water parameters did not differ appreciably from those reported for the fluoride toxicity bioassay.

Mean values of water quality parameters analyzed during fluoride toxicity bioassay are presented in Table 2. All values are within water quality criteria for aquatic organisms (26). Ammonia and nitrite were not detected at the start, and no precipitation of fluoride salts was observed during these bioassays.

Mean concentrations of fluoride and sodium, conductivity, and the mortality

Table 1

Mean Values of Water Parameters (Means ±S.D., n = 4) and Species Mortality Obtained During NaCl Toxicity Tests.

Parameter	Salmo gairdneri	Salmo trutta fario
Water Temperature (°C)	15.6 ±0.07	15.9 ±0.14
Alkalinity (ppm $CaCO_3$)	37.1 ±1.91	29.2 ±1.27
Hardness (ppm $CaCO_3$)	22.3 ±1.16	21.7 ±1.72
Dissolved oxygen (ppm)	10.4 ±0.14	10.4 ±0.07
Chlorine (ppm Cl_2)	N.D.	N.D.
Ammonia (ppm N)	0.13 ±0.170	0.08 ±0.110
Nitrite (ppm N)	0.01 ±0.010	0.01 ±0.006
Fluoride (ppm F^-)	0.09 ±0.005	0.08 ±0.011
Potassium (ppm K^+)	0.11 ±0.060	0.12 ±0.010
pH	7.58 ±0.060	7.64 ±0.040
Chloride (ppm)	511.4 ±26.9	608.7 ±27.3
Sodium (ppm Na^+)	299.5 ±21.9	379.0 ±15.6
Conductivity (µmhos/cm)	705.0 ±21.2	1175.0 ±35.4
Mortality (%)	N.D.	N.D.

N.D. = Not Detected.

Table 2

Mean Values of Water Quality Parameters (Means ±S.D., n = 24) Analyzed During NaF Toxicity Bioassays.

Parameter	Salmo gairdneri	Salmo trutta fario
Water Temperature (°C)	15.3 ±0.22	16.1 ±0.13
Alkalinity (ppm $CaCO_3$)	37.5 ±2.09	32.2 ±1.92
Hardness (ppm $CaCO_3$)	22.4 ±1.79	21.2 ±2.55
Chlorine (ppm Cl_2)	N.D.	N.D.
Chloride (ppm)	10.0 ±1.22	10.8 ±0.43
Dissolved oxygen (ppm)	10.1 ±0.28	10.1 ±0.20
Ammonia (ppm N)	0.15 ±0.157	0.12 ±0.121
Nitrite (ppm N)	0.01 ±0.009	0.01 ±0.008
Potassium (ppm K^+)	0.08 ±0.020	0.14 ±0.016
pH	7.58 ±0.179	7.63 ±0.185

N.D. = not detected

obtained during fluoride short-term toxicity bioassays are presented in Table 3. Standard deviations were lower than 10% of their respective mean values. There was no mortality in control aquaria. For the experimental group, the mortality increased with increase in fluoride concentrations. Fry in the fluoride aquaria showed hyperexcitability and hyperventilation at first and, at alternate times during test, hypoexcitability, darkened backs and a decrease in respiration before their death. However, these sublethal symptoms were not observed in fry exposed to a mean concentration of 22.3 ppm F^-.

The LC_{50} values at 96, 120, 144, 168 and 192 hours, their 95% confidence limits, and χ^2 values obtained for each test trout species are presented in Table 4. All χ^2 values were lower than those for $p = 0.005$, indicating that the data are not significantly heterogeneous. The Maximum Acceptable Toxic Concentration (MATC) after 8 days' exposure to fluoride solutions was 27.6 ppm F^- for rainbow trout.

The significant differences between LC_{50} values are shown in Table 4. LC_{50} values for brown trout were significantly ($p < 0.05$) higher than those for rainbow trout to sodium fluoride for 96, 120, 144 and 192 hours. This indicates that Salmo gairdneri is a more sensitive species to F^- ions than Salmo trutta fario.

Discussion

Although it has already been indicated that among the metallic ions, Na^+ ion has the lowest toxicity for aquatic organisms (27), this study has demonstrated the toxic effect of sodium fluoride on trout species is fundamentally due to fluoride ions.

On the other hand, rainbo more resistant to fluoride io since Camargo and Tarazona for F^- in soft water to be 26.3 bulbifera, H. exocellata, H. p larvae, respectively.

Because a range of widely fluoride in fish and a direct co ent studies is not fit because

Mean Values of Conductivity, S NaF Toxicity Bioassays for Rai c = control aquarium; 1, 2, 3,

Salmo gairdneri	c-c	
Conductivity (µmhos/cm)	32.5-30.0	14
Sodium (ppm Na^+)	4.8-5.1	26.
Fluoride (ppm F^-)	0.08-0.09	22.
Mortality 96 hrs	0-0	
120 hrs	0-0	
144 hrs	0-0	
168 hrs	0-0	
192 hrs	0-0	
Salmo trutta fario	**c-c**	
Conductivity (µmhos/cm)	42.5-40.0	2
Sodium (ppm Na^+)	7.3-8.3	4
Fluoride (ppm F^-)	0.08-0.08	3
Mortality 96 hrs	0-0	
120 hrs	0-0	
144 hrs	0-0	
168 hrs	0-0	
192 hrs	0-0	

On the other hand, rainbow trout and brown trout appear significantly more resistant to fluoride ion than freshwater benthic microinvertebrates, since Camargo and Tarazona (9) have estimated the 96 hour LC_{50} values for F^- in soft water to be 26.3, 26.5, 38.5, 48.2 and 44.9 ppm for Hydropsyche bulbifera, H. exocellata, H. pellucidula, H. lobata, and Chimarra marginata larvae, respectively.

Because a range of widely divergent LC_{50} values has been reported for fluoride in fish and a direct comparison of LC_{50} values obtained during different studies is not fit because of the several methods used to report toxic

Table 3

Mean Values of Conductivity, Sodium and Fluoride Obtained in Duplicate During NaF Toxicity Bioassays for Rainbow Trout and Brown Trout.
c = control aquarium; 1, 2, 3, 4 and 5 = fluoride aquaria

Salmo gairdneri	c-c	1-1	2-2	3-3	4-4	5-5
Conductivity (µmhos/cm)	32.5-30.0	142-145	202-202	325-335	485-495	635-640
Sodium (ppm Na^+)	4.8-5.1	26.1-26.4	46.3-44.1	69.7-71.1	114-113	190-190
Fluoride (ppm F^-)	0.08-0.09	22.3-22.3	34.4-34.2	57.6-57.3	91.4-91.0	144-146
Mortality 96 hrs	0-0	0-0	10-10	20-30	50-30	60-70
120 hrs	0-0	0-0	10-10	20-30	50-40	70-80
144 hrs	0-0	0-0	10-10	20-30	60-40	70-90
168 hrs	0-0	0-0	20-10	20-30	60-60	90-90
192 hrs	0-0	0-0	20-10	20-40	70-70	90-100
Salmo trutta fario	c-c	1-1	2-2	3-3	4-4	5-5
Conductivity (µmhos/cm)	42.5-40.0	218-213	318-315	485-495	650-665	1075-1025
Sodium (ppm Na^+)	7.3-8.3	48.2-48.8	68.2-69.3	111-113	189-190	293-291
Fluoride (ppm F^-)	0.08-0.08	33.9-35.0	55.4-53.8	90.3-90.6	146-150	236-228
Mortality 96 hrs	0-0	0-0	10-0	20-20	40-30	70-80
120 hrs	0-0	0-0	10-0	20-30	50-30	80-90
144 hrs	0-0	0-0	10-10	30-30	60-40	80-90
168 hrs	0-0	0-10	10-10	30-40	60-50	100-90
192 hrs	0-0	10-10	20-10	40-40	60-50	100-100

Table 4

LC_{50} values, their 95% confidence limits and χ^2 values obtained for each test trout species.
X = rainbow trout; Y = brown trout

		LC_{50} (ppm F^-)	95% c.l. (ppm F^-)	χ^2 values
96 hrs	X	107.5	138.0-83.7	2.53
	Y	164.5*	205.1-131.9	3.41
120 hrs	X	92.4	116.0-73.6	1.47
	Y	135.6*	161.2-114.0	5.40
144 hrs	X	85.1	106.5-68.0	2.88
	Y	118.5*	149.2-94.1	4.79
168 hrs	X	73.4	96.4-55.9	2.90
	Y	105.1*	134.9-81.8	6.63
192 hrs	X	64.1	82.2-50.0	3.15
	Y	97.5*	123.8-76.8	6.90

* $p < 0.05$.

effects, maximum safe criteria of fluoride ion for fish in natural ecosystems have not yet been achieved (26). However, it is evident that rainbow trout and other species of freshwater fish may bear higher fluoride concentrations in hard water than in soft water (12,20). In this sense, it has been reported (16) that fluoride ion may form stable complexes with calcium in blood and bone, and Pimental and Bulkley (20) have suggested that a reservoir of calcium in the water surrounding fish tends to compensate for this loss of calcium and thereby delays toxic effects of fluoride on the organism.

Further research on the toxicity of fluoride ion to freshwater fish should, therefore, be conducted under conditions of highest toxicity, namely, soft water, for obtaining a suitable safe level of F^-. The estimated 8-day MATC might furnish a preliminary safe criterion for trout species. Nevertheless, chronic toxicity bioassay is needed to improve fluoride quality criteria. To this end, the data obtained from the present work may provide the background for future long-term toxicity tesearch to establish safe fluoride standards for freshwater fish.

References

1. Dobbs, C.G.: Fluoride and the Environment. **Fluoride**, 7:123-135, 1974.
2. Dave, G.: Effects of Fluoride on Growth. Reproduction and Survival in Daphnia magna. **Comp. Biochem Physiol.**, 78C:425-431, 1984.
3. Martin, J.M. and Salvadori, F.: Fluoride Pollution in French Rivers and Estuaries. **Estuarine, Costal and Shelf Science**, 17:231-242, 1983.
4. McClurg, T.P.: Effects of Fluoride, Cadmium and Mercury on the Estuarine Prawn Penaeus indicus. **Water SA**, 10:39-45, 1984.
5. Oliveira, L., Antia, N.J. and Bisalputra, T.: Culture Studies on the Effects from Fluoride Pollution on the Growth of Marine Phytoplankters. **J. Fish Res. Board Can.**, 35:1500-1504, 1978.

6. LeBlanc, G.A.: Acute Toxic magna). **Bull. Environm. To**
7. Pankhurst, N.W., Boyden, C Effluent on Marine Organi 312, 1980.
8. Hemens, J. and Warwick, Organisms. **Wat. Res.**, 6:13
9. Camargo, J.A. and Taraz Larvae. **Proceedings of Is** Copenhagen, Denmark, [in
10. Hemens, J., Warwick, R.J. to Low Fluoride Concent **in Water Tech.**, 7:579-585,
11. Milhaud, L., Bahri, E. a in Gabes Gulf. **Fluoride**, 1
12. Smith, L.R., Holsen, T.M. Studies on the Acute To Minnow, and Rainbow Trou
13. Chitra, T., Reddy, M.M. Liver Tissue Enzymes in C 16:48-51, 1983.
14. Pillai, K.S. and Mane, U. and Minerals in Fry of Ca
15. Angelovic, J.W., Sigler, W. in Rainbow Trout. **J. Wate**
16. Sigler, W.F. and Neuhold, **J. Wildl. Dis.**, 8:252-254,
17. Neuhold, J.M. and Sigler, Rainbow Trout. **Trans. Am**
18. Herbert, D.W.N. and Shu Trout. **Water and Waste T**
19. Vallin, S.: The Toxicity of
20 Pimental, R. and Bulkley Toxicity to Rainbow T 2:381-386, 1983.
21. APHA, AWWA, WPCF: S **and Wastewater.** Americ Assoc., Water Pollution C
22. Rodier, J.: **Análisis de las**
23. USEPA: **Methods for Ac and Amphibians.** U.S. Washington, D.C., 1975.
24. Litchfield, J.T. and Wilco Effect Experiments. **J. Ph**
25. Buikema, A.L., Niederlel Part IV – Toxicity Testin
26. USEPA: **Criteria for** 440/5-86-001, Washington,
27. Hellawell, J.M.: **Biologic mental Management.** E New York, 1986.

6. LeBlanc, G.A.: Acute Toxicity of Priority Pollutans to Water Flea (Daphnia magna). **Bull. Environm. Toxicol.**, 24:684-691, 1980.
7. Pankhurst, N.W., Boyden, C.R. and Wilson, J.B.: The Effect of a Fluoride Effluent on Marine Organisms. **Environmental Pollution** (Series A), 23:299-312, 1980.
8. Hemens, J. and Warwick, R.J.: The Effects of Fluoride on Estuarine Organisms. **Wat. Res.**, 6:1301-1308, 1972.
9. Camargo, J.A. and Tarazona, J.V.: Fluoride Toxicity to Aquatic Insect Larvae. **Proceedings of 1st European Conference on Ecotoxicology**, 1988 Copenhagen, Denmark, [in press].
10. Hemens, J., Warwick, R.J. and Oliff, W.D.: Effect of Extended Exposure to Low Fluoride Concentration in Estuarine Fish and Crustacea. **Progr. in Water Tech.**, 7:579-585, 1975.
11. Milhaud, L., Bahri, E. and Dridi, A.: The Effects of Fluoride on Fish in Gabes Gulf. **Fluoride**, 14:161-168, 1981.
12. Smith, L.R., Holsen, T.M., Ibay, N.C., Block, R.M. and De Leon, A.B.: Studies on the Acute Toxicity of Fluoride Ion to Stickleback, Fathead Minnow, and Rainbow Trout. **Chemosphere**, 14:1383-1389, 1985.
13. Chitra, T., Reddy, M.M. and Ramana Rao, J.V.: Levels of Muscle and Liver Tissue Enzymes in Channa punctatus Bloch Exposed to NaF. **Fluoride**, 16:48-51, 1983.
14. Pillai, K.S. and Mane, U.H.: Effect of F^- Effluent on Some Metabolites and Minerals in Fry of Catla catla (Hamilton). **Fluoride**, 17:224-233, 1984.
15. Angelovic, J.W., Sigler, W.F. and Neuhold, J.M.: Temperature and Fluorosis in Rainbow Trout. **J. Water Pollut. Control Fed.**, 33:371-381, 1961.
16. Sigler, W.F. and Neuhold, J.M.: Fluoride Intoxication in Fish: A Review. **J. Wildl. Dis.**, 8:252-254, 1972.
17. Neuhold, J.M. and Sigler, W.F.: Effects of Sodium Fluoride on Carp and Rainbow Trout. **Trans. Am. Fish Soc.**, 89:358-370, 1960.
18. Herbert, D.W.N. and Shurben, D.S.: The Toxicity of Fluoride to Rainbow Trout. **Water and Waste Treatment**, 10:141-142, 1964.
19. Vallin, S.: The Toxicity of Fluoride to Fish. **Vatten**, 24:51-58, 1968.
20 Pimental, R. and Bulkley, R.V.: Influence of Water Hardness on Fluoride Toxicity to Rainbow Trout. **Environmental Toxicology and Chemistry**, 2:381-386, 1983.
21. APHA, AWWA, WPCF: **Standard Methods for the Examination of Water and Wastewater.** American Public Health Assoc., American Water Works Assoc., Water Pollution Control Fed., 15th ed., Washington D.C., 1980.
22. Rodier, J.: **Análisis de las Aguas.** Omega ed., Barcelona, 1981.
23. USEPA: **Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians.** U.S. Environmental Protection Agency, 600/3-75-009, Washington, D.C., 1975.
24. Litchfield, J.T. and Wilcoxon, F.: A Simplified Method of Evaluating Dose-Effect Experiments. **J. Pharmacol. Exp. Ther.**, 96:99-113, 1949.
25. Buikema, A.L., Niederlehner, B.R. and Cairns, J.: Biological Monitoring Part IV — Toxicity Testing. **Water Res.**, 16:239-262, 1982.
26. USEPA: **Criteria for Water.** U.S. Environmental Protection Agency, 440/5-86-001, Washington, D.C., 1986.
27. Hellawell, J.M.: **Biological Indicators of Freshwater Pollution and Environmental Management.** Elsevier Applied Science Publishers, London and New York, 1986.

****