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Fluorides in Wastewater Discharges: Toxic Challenges to the St. Lawrence River Biological Community

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This literature review examines the risk to fluvial organisms of fluoride released to the St. Lawrence River by the Montréal Urban Community wastewater treatment plant. The following key points are presented regarding the behaviour of fluoride in aquatic systems: fluoride is not removed by primary sewage treatment methods; fluoride from the treatment plant is rapidly diluted to background levels; aquatic plants do not accumulate significant levels of fluoride; fluoride is generally sequestered in the shell/exoskeleton/skeleton and skin of animals and released via the urinary system; fluoride ion is not very toxic in aquatic systems; there is rarely a large difference between acute and chronic (or lethal and sublethal) threshold levels of fluoride in aquatic systems; the most sensitive aquatic organisms are trout and fingernail clams; finally, the only significant evidence of synergistic action is with aluminium. It is concluded that fluoridation of the city of Montréal's drinking water would not pose any significant additional risk to the biological community in the receiving waters of the St. Lawrence River.

Introduction

Drinking water fluoridation has been a contentious issue since it was first introduced in North America in 1945, and remains so each time a municipality considers it (Moolenburgh 1987; Belair and Viau 1988; Colquhoun 1994). This report has its origins in the public consultations conducted by the city of Montréal concerning a possible fluoridation project. Questions were raised concerning the risk of impact on the biological community of the St. Lawrence River downstream from the outfall of the Montréal sewage treatment plant, due to the resultant low levels of fluoride (Ville de Montréal 1988). Subsequent studies at the treatment plant indicated considerable fluctuation in existing daily and seasonal fluoride levels (Gehr and Leduc 1992; Choueiri et al. 1995), partially due to industrial discharges.

In conducting an ecotoxicological risk assessment, it is necessary to examine three types of information (Pascoe 1993). (1) Ecological: the organisms and communities present that may be or have been impacted by the presence of the compound under study. (2) Chemical: the properties of the compound which affect its presence and movement within the ecosystem. (3) Toxicological: the nature and degree of dysfunction caused

by the compound's contact with the organisms of concern. An evaluation of risk can then be made by determining the availability of the compound and its toxicity to the ecosystem due to exposure over time.

The structure of this review is thus an examination of the biological resources present, the chemical and physical properties of fluoride, its abiotic transport and distribution, its toxicokinetics (the absorption, accumulation, biotransformation and elimination of fluoride within target organisms), and its toxicodynamics (effective concentrations and mechanisms of action). At the end of each section, the key point(s) relevant to the assessment of risk are highlighted. Having presented all this information, the final section evaluates the risk of damage. The conclusions have been expanded to cover possible consequences of the total fluoride load rather than just the additional loading consequent to water fluoridation by the city of Montréal.

It is important to be aware that fluoride is also considered to be a significant air pollutant, arising primarily from aluminum and phosphate production plants. Consequently, much of the information pertaining to toxicological effects is based on terrestrial systems (e.g., WHO 1985; CCE 1986; Government of Canada 1993). Where possible, data have been extrapolated to the aquatic environment.

Biological Resources in the Area of Study

Because of rapid currents and heavy shipping traffic in the immediate receiving waters of Montréal's municipal effluent discharge, the community of organisms likely to come into contact with elevated fluoride levels in the treatment plant's plume (Fig. 1) is small. Pilon et al. (1980) have reported that at the river border next to Île Sainte-Thérèse, there exists an annual community of submerged macrophytes, primarily filamentous *Potamogeton pectinatus*, and some grass-like *Alisma gramineum*. Close to shore, there are emergent bullrushes (*Typha angustifolia*) and reeds (*Scirpus americanus*, *S. acutus* and *S. validus*). This zone of plant growth is not stable or calm enough to serve as a spawning ground or "nursery" for local fish species, although older fish may occasionally use the weed bed as cover.

The only benthic organisms identified were immature oligochaete worms (Tubificidae), considered indicators of polluted water, and one species of amphipod, *Gammarus gammarus*. The density of individuals, 52/m^2, and the index of species diversity, 0.81, are considered low. Nothing was reported concerning populations of bacteria, algae or protozoans, the organisms of "biological self-purification" that form the basis of the food chain.

Fish species common to this sector of the St. Lawrence River generally prefer quieter waters to those of the receiving waters, although they may venture into the plume of the municipal waste from time to time. They include northern pike (*Esox lucius*), yellow perch (*Perca flavescens*),

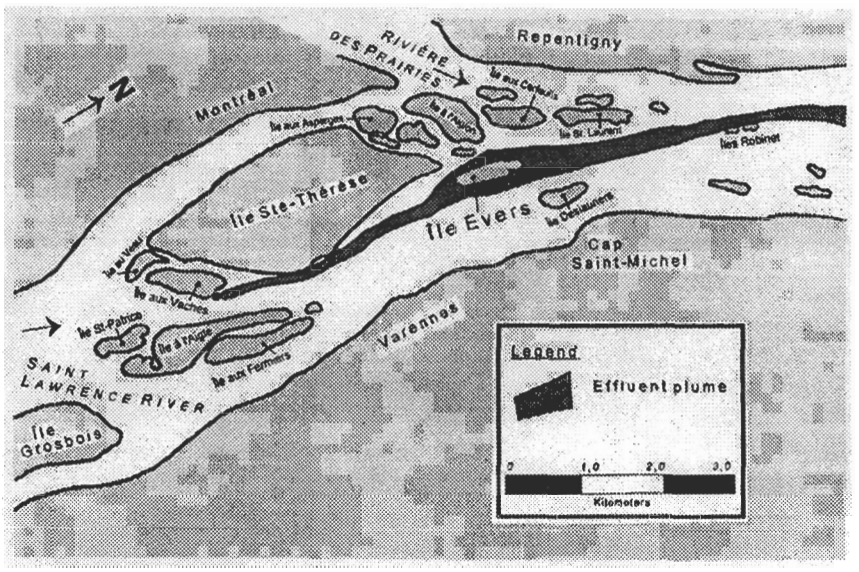

Fig. 1. Map of the area of study showing a typical effluent plume, as defined by rhodamine studies (adapted from Boulanger 1984).

largemouth bass (*Micropterus salmoides*), brown bullhead (*Ictalurus nebulosus*), golden shiner (*Notemigonus crysolencas*), carp (*Cyrinus carpio*), pumpkinseed (*Lepomis gibbosus*), black crappie (*Pomoxis nigromaculatus*), bowfin (*Amia calva*), and central mudminnow (*Umbra limi*) (Pilon et al. 1980). Also, both the eel (*Anguilla bostoniensis*) and sea lamprey (*Petromyzon marinus*) use the St. Lawrence as a migratory route (Hubbs and Lagler 1964) and may pass through this area, although no official record of them in the zone of concern was found.

Some members of the bird population may be at risk, particularly the shorebirds and surface-feeding ducks that consume plants and small invertebrates living in the outflow plume. Fish-eating species may be at lower risk, as their prey is probably less regularly exposed to the fluoride-bearing plume. Table 1 shows the species with aquatic feeding habits that have been observed in the area. Of note is the small colony of rare Wilson's phalarope (*Steganopus tricolor*) that nests on Île Evers, directly in the path of the plume. Île Sainte-Thérèse and smaller downstream islands are important rest sites on the migratory routes of many other species (Pilon et al. 1980).

The only local mammal likely to be at risk is the muskrat (*Ondatra zibethica*), which spends much of its time in the water and whose food source is primarily aquatic. Raccoons (*Procyon lotor*) might conceivably be exposed if the aquatic proportion of their diet is large.

This area was considered polluted before the treatment plant commenced operation (Gravel and Pageau 1979). The effect of removing numerous raw sewage outlets upstream and replacing them with the single,

Table 1. List of the bird species with aquatic feeding habits that have been observed in the area of study (adapted from Pilon et al. 1980, feeding habits from Peterson 1980)

Common name	Scientific name	Food
Pied-billed Grebe	*Podilymbus podiceps*	Small fish, invertebrates, tadpoles
Double-crested Cormorant	*Phalacrocorax auritus*	Fish, crustaceans
Great Blue Heron	*Ardea herodias*	Fish, amphibians,crayfish, insects
Green Heron	*Butorides striatus*	Fish, amphibians, invertebrates
Snowy Egret	*Egretta thula*	Fish, amphibians, invertebrates
Black-crowned Night Heron	*Nycticorax nycticorax*	Fish, amphibians, invertebrates
American Bittern	*Botaurus lentiginosus*	Fish, amphibians, invertebrates
Glossy Ibis	*Plegadis falcinellus*	Small fish, invertebrates
Canada Goose	*Branta canadensis*	Vegetation
Snow Goose	*Chen caerulescens*	Vegetation
Mallard Duck	*Anas platyrhynchos*	Vegetation, invertebrates
Black Duck	*Anas rubripes*	Vegetation, invertebrates
Gadwall	*Anas strepera*	Vegetation, invertebrates
American Widgeon	*Anas americana*	Vegetation, invertebrates
Common Pintail	*Anas acuta*	Vegetation, invertebrates
Green-winged Teal	*Anas crecca*	Vegetation, invertebrates
Blue-winged Teal	*Anas discors*	Vegetation, invertebrates
Northern Shoveler	*Anas clypeata*	Vegetation, invertebrates
Osprey	*Pandion haliaetus*	Fish
Common Gallinule	*Gallinula chloropus*	Invertebrates
Killdeer	*Charadrius vociferus*	Invertebrates, vegetation
Common Snipe	*Capella gallinago*	Invertebrates, vegetation
Spotted Sandpiper	*Actitis macularia*	Invertebrates
Greater Yellowlegs	*Tringa melanoleuca*	Invertebrates
Lesser Yellowlegs	*Tringa flavipes*	Invertebrates
Least Sandpiper	*Calidris minutilla*	Invertebrates
Dunlin	*Calidris alpina*	Invertebrates
Sanderling	*Calidris alba*	Invertebrates
Marbled Goodwit	*Limosa fedoa*	Invertebrates
Wilson's Phalarope	*Steganopus tricolor*	Insect larvae, invertebrates, plankton
Greater Black-backed Gull	*Larus marinus*	Fish, invertebrates, vegetation, carrion
Herring Gull	*Larus argentatus*	Fish, invertebrates, vegetation, carrion
Ring-billed Gull	*Larus delawarensis*	Fish, invertebrates, vegetation, carrion
Bonaparte's Gull	*Larus philadelphia*	Fish, invertebrates, vegetation, carrion
Common Tern	*Sterna hirundo*	Small fish, large invertebrates
Black Tern	*Chlidonias niger*	Small fish, large invertebrates
Belted Kingfisher	*Megaceryle alcyon*	Fish

treated sewage outlet at this location is difficult to state. The plants and benthic organisms in the most highly impacted zone are pollution-tolerant species. They may still be at some risk of fluoride accumulation and toxic effects. The remaining animals in the area are unlikely to spend much time in the treatment plant plume, as it is in a highly turbulent area. Direct exposure will thus be minimized for such organisms, and their food sources will be the main route of exposure, assuming that fluoride is subject to biomagnification.

Key point: The biological resources at risk of direct effects by fluoride are minimal.

Chemical and Physical Properties

The fluoride ion is the smallest of the halides (1.33 angstroms ionic radius, 19.00 g/mole molecular weight) and, as such, the most electronegative (Bank and Goldwhite 1966; Weast 1984). It has the potential to be highly reactive, either by attracting an electron to fill the 2p valency shell or, less commonly, by donating an electron pair in an unsaturated system. It forms strikingly strong hydrogen bonds with water (Diercksen et al. 1975), carboxylates and amides (Emsley et al. 1981) as well as very stable polar covalent bonds with carbon. The facility for hydrogen bond formation explains the stability of the ion in aqueous solution, where it holds five water molecules per ion, and its high hydration energy (the amount of energy required to remove the ion from water). Its behaviour in real and model biological systems differs distinctly from other halides and most polyatomic anions (Wright and Diamond 1977). Because of its small ionic radius, it potentially has a high capacity to denature proteins and other essential biological molecules (Wiseman 1970). These features justify a concern for the welfare of organisms exposed to fluoride.

Key point: The fluoride ion in solution forms stable hydrogen bonds with five water molecules.

Transport and Distribution

Much research has been done on the transport and distribution of gaseous and particulate fluorides in air (e.g., Ouellet et al.1983), and some research exists on high concentrations of fluoride released in industrial effluents (e.g., Damkaer and Dey 1989; Camargo 1992), but municipal sources such as this study examines have generally been ignored (Davies 1989; Foulkes and Anderson 1994). The work that has been done indicates that (1) primary sewage treatment does not remove fluorides from water (Gehr and Leduc 1992), although secondary treatment can reduce concentrations by as much as 70% (Masuda 1964); (2) the concentration of fluoride (and most other contaminants) entering the receiving waters of the St. Lawrence River is diluted to the background level of 0.2 mg F^-/L

(Choueiri et al. 1995) within 2 km (Boulanger 1984; Chu et al. 1991) (smaller, cleaner rivers may, however, be significantly affected even 20 km downstream [Camargo et al. 1992b]); (3) half the fluoride added to quiet waters is removed to other environmental compartments, mostly to sediment, in 0.6 day (Kudo and Garrec 1983); (4) fluoride levels in sewage are elevated even when water is not fluoridated (industries discharging into municipal sewage systems as well as runoff from contaminated rain and snow can make considerable contributions [Ouellet et al. 1983; Choueiri et al. 1995]); and (5) the fluoride flux in the St. Lawrence River would increase by 1.2% if Montréal were to fluoridate its drinking water (Gehr and Leduc 1992).

Fluoride ions may be removed from water when that water is applied to soil by processes similar to those used in wastewater treatment (e.g., Patterson 1985). This is of interest since drinking water is also used for such purposes as watering gardens and lawns, and washing vehicles and buildings. The runoff eventually reaches the treatment plant via storm sewers. At the interface between water and soil, fluoride reacts in a variety of competing chemical equilibria dependent on pH, temperature, particulate organic carbon, clay content (Pickering 1985; Baars et al. 1987) and basic soil type (Wilke 1987). A simple statement about the fate and effects of fluorides in soil is therefore difficult. Two potential events are worth noting, though. Calcium fluoride (CaF_2) may arise where high calcium-based substrates exist, but this formation requires a fluoride ion concentration of at least 9.5 mg/L, almost 10 times higher than levels achieved in drinking water. Gibson et al. (1992) showed that approximately 75% of fluoride applied to an oxisol was simply sorbed by the soil particles. However, aluminium ions, soluble organo-aluminium complexes and alumino-fluoride compounds of the general formula $[AlF_n(H_2O)_{6-n}]^{(3-n)+}(n=1\text{-}6)$ are released from clays and organic matter at low fluoride concentrations. This mobilization follows hydroxyl replacement and subsequent crystal lattice breakdown of substrates in the presence of waterborne fluoride (Slavek et al. 1984; Elrashidi and Lindsay 1987; Pickering et al. 1988; Gibson et al. 1992). The release of aluminium from soil particles in the form of soluble complexes, a fluoride property, may be of concern in that it may contribute to a joint effect in waters already receiving enhanced levels of aluminium as a consequence of acid precipitation (Neal 1989). The aluminium-mobilizing action of fluoride may also apply to St. Lawrence River sediments, where aluminium levels of 60,000 μg/g and more have been detected, and to suspended solids in the water column, where aluminium concentrations may reach 1.41 mg/L (Sloterdijk 1988).

Water quality characteristics which may have a significant effect on the fate of fluorides include hardness or alkalinity (as $CaCO_3$), pH and temperature. As noted for soils, calcium may precipitate fluoride ions as insoluble CaF_2. The St. Lawrence River in the area of the receiving waters has been reported to have a hardness in the range of 52 to 105 mg/L as $CaCO_3$ (moderately hard). pH is an important factor affecting the speci-

ation of total fluoride present. The St. Lawrence has a pH of 7.8 to 8.1 (Sloterdijk 1988). Temperatures in the receiving waters range from 0°C in winter to 20°C in summer. The level of suspended solids in the effluent, as well as in the river, is also a factor, as fluoride ions may be adsorbed by organic material or fine clay particles. These materials, as they settle out, can contribute considerably to fluoride levels in sediments (Gordon and Tourangeau 1977; Kudo and Garrec 1983).

Key points: (i) Fluoride is not removed by primary sewage treatment methods. (ii) Fluoride entering the St. Lawrence at the treatment plant outlet is rapidly diluted to background levels. (iii) Fluoride ions added to soil or sediment may mobilize aluminium in the form of soluble complexes.

Toxicokinetics

Adsorption/Accumulation/Elimination

Plants

Terrestrial plants have been shown to accumulate fluoride, mainly by adsorption through the leaves from gaseous and particulate air pollution, although some uptake occurs through the roots from contaminated soils (Weinstein 1977; Andrews et al. 1989; Machoy and Machoy-Mokrzyńska 1990). Fluorides can also be absorbed from irrigation water, particularly if it is sprayed on leaves, and directly from the water in flooded salt marshes (Baars et al. 1987) and in hydroponic systems, as fluoride ions can diffuse easily across epithelial membranes (Weinstein 1977).

There are fewer studies available on the fate of fluorides in aquatic plants. Freshwater algae and plants do accumulate fluorides but generally at rates much below that of terrestrial plants, and steady-state tissue concentrations of fluorides appear to be reached quickly (Bundock et al. 1982). Chaisemartin (1985) reported a plateau in the rate of fluoride fixation in the macrophyte *Rannuculus aquatilis* exposed to 10 mg F^-/L, after 8 hours. This aquatic species is known to be pollution-tolerant and is thought to effectively depurate fluoride, a capacity not generally available to terrestrial plants. Duckweed (*Spirodela polyrrhiza*) growing in freshwater ponds with fluoride concentrations between 0.5 and 1.0 mg/L had a fluoride content of roughly 35 μg/g dry weight, as did fronds from unpolluted waters used in laboratory studies. Significant accumulation occurred at and above 5 mg F^-/L, reaching plateau levels 40 times the exposure concentration in 3 to 5 days (Shire and Chandra 1991). The aquatic liverwort *Scapania undulata* increased fluoride content 21 to 67% when exposed to 250 mg F^-/L. The actual degree of accumulation was dependent on the fluoride level in the water from which the liverwort had been collected, with less fluoride being accumulated by those plants from sources with higher fluoride concentrations (Samecka-Cymerman and Kempers 1990).

Nichol et al. (1987) found that the cells of the chlorophyte algae *Chlorella pyrenoidosa* did not take up fluoride even at 150 mg F^-/L. A cyanophyte species of algae, *Synechococcus leopoliensis*, was found to accumulate fluoride temporarily at pH 6.26 but not at pH 6.88. Fluoride was released rapidly (half-life 10 to 20 min) when cells were placed in fluoride-free growth medium. When pre-exposed to nontoxic levels of fluoride, *S. leopoliensis* was shown to adapt, possibly by increasing the permeability of the cell wall to F^-, thereby reducing or preventing accumulation on subsequent exposure to normally toxic levels (Nichol et al. 1987).

While some marine algal species do appear to accumulate fluoride even at the normal 1.0 mg F^-/L level in seawater (Groth 1975), Hemens and Warwick (1972) found no accumulation by either *Cladophora* algae or eel grass (*Zostera capensis*) at 52 mg F^-/L for 72 days.

It has been suggested that fluoride initially enters aquatic plant cells or crosses cellular membranes as the molecule HF. The uptake of waterborne fluoride has been shown to increase for some algae at lower ambient pH, where the concentration of HF is higher (Nichol et al. 1987).

Key point: Aquatic plants do not accumulate significant levels of fluoride, either because they do not absorb the hydrated ion or because they can effectively depurate it.

Invertebrates

It has been inferred that terrestrial invertebrates accumulate high body burdens of fluoride from the food chain (i.e., Buse 1986). In the aquatic environment, Gordon and Tourangeau (1977) reported elevated, although declining, fluoride levels in aquatic insects (up to 243 μg F^-/g) even 9 years after the shutdown of a phosphate plant that had discharged effluent to the stream. The water level was only 0.2 mg F^-/L, but sediments still contained large amounts of fluoride, supporting the biomagnification concept. Chaisemartin (1985) studied an experimental food chain with a pollution-tolerant macrophyte, an herbivorous snail (*Lymnaea peregra*) and its predator, a crayfish (*Oronectes limosus*). Bioaccumulation ratios were low, only 0.49 to 0.86 for the crayfish at 10 mg F^-/L exposure, and snails artificially loaded with fluoride seemed to contribute little to the total body burden of the crayfish. Levels of fluoride in the crayfish exoskeleton were 6 to 10 times higher than those in gills or muscles, with greater accumulation noted in warmer temperatures. Unlike the algae, previously contaminated organisms accumulated up to 10 times more fluoride than ones from clean environments. It was concluded that accumulation involved the complex interaction of biotic and abiotic factors.

In a preliminary study, Mane and Pillai (1985) found no significant correlation between fluoride concentration in river water (0.47 to 0.58 mg F^-/L) and fluoride levels in the flesh of three bivalve mollusc species living in the river. Unfortunately, they did not determine levels in the shells, but flesh levels were quite low, less than 0.05 μg F^-/g (wet weight). They did note a possible relationship between body length (and therefore vol-

ume of water processed) and fluoride content. Kudo and Garrec (1983) reported bioaccumulation factors of 11 to 85 in molluscs exposed to ammonium fluoride in an experimental pond. The fluoride had a biological half-life of 43 days.

Fluoride kinetic studies on marine and estuarine invertebrates have been more extensive than in fresh water, but because fluoride levels in seawater are naturally in the 1 mg F^-/L range and the organisms are physiologically adapted to a high halide environment, the conclusions drawn may be suspect when applied to freshwater systems. Hemens and Warwick (1972) found that mud crab (*Tylodiplax blephariskios*), sand shrimp (*Palaemon pacificus*) and prawn (*Penaeus indicus*) accumulate fluoride primarily from the water, with factors of whole body accumulation in 52 mg F^-/L water ranging from 8 to 30 times. Wright and Davison (1975) confirmed this for crab (*Carcinus maenas*) at 30 mg F^-/L (10–25X), although accumulations were much lower at 10 mg F^-/L and negligible at 1 mg F^-/L. They distinguished between storage in the exoskeleton and buildup in various soft tissues, with muscle and haemolymph simply reflecting ambient fluoride levels. Interestingly, a short-term experiment with mussels (*Mytilus edulis*) showed a similar accumulation factor in the shell (20X), but the soft tissue concentration increased by a factor of roughly 100, with levels exceeding that of the shell in some cases. They suggested that this unusual pattern is due to the large volumes of seawater processed by these sessile filter feeders. Barbaro et al. (1981) also found molluscs to accumulate fluoride far above ambient levels (20–70X) in soft tissues, with a clear relationship between exposure level and tissue concentration.

Moore (1971) provided detailed information on fluoride kinetics in the blue crab (*Callinectes sapidus*), demonstrating preferential uptake by the exoskeleton in terms of both absolute levels and rate of uptake, in comparison to gills, hepatopancreas and muscle. At 2 mg F^-/L for 30 days, however, even the exoskeleton did not show significant concentration. Fluoride release, once the crabs were removed from exposure, was only slightly slower than uptake and occurred in all tissues, including the exoskeleton. McClurg (1984) reported similar results for the prawn (*Penaeus indicus*) and suggested that the shedding of the exoskeleton (ecdysis) may be a mechanism for reducing fluoride content. Antarctic krill accumulate as much as 3,300 μg F^-/g in the cuticle but less than 1 μg F^-/g in the other tissues. When the krill is killed, however, the fluoride rapidly translocates from the cuticle to the soft tissues (Landy et al. 1991).

Key points: (i) Invertebrates generally cope effectively with fluoride by accumulating it in the exoskeleton or shell. (ii) Ecdysis provides invertebrates with a means of eliminating excess fluoride completely.

Fish

Neuhold and Sigler (1960) demonstrated that the fluoride uptake rate in rainbow trout (*Salmo gairdneri*) and carp (*Cyrinus carpio*) was related to fluoride content in water as well as duration of exposure. In muscle

tissue, low exposure levels in soft water produced an average concentration of 2.95 μg F^-/g, but the presence of small bones in the flesh led to high variability in determinations. Exposure to higher levels (i.e., 25 mg F^-/L) produced a sevenfold increase in tissue concentrations. In osseous tissue (cancellous and skeletal bone), the double reciprocal plot of uptake rate (μg F^-/ g/hour) versus medium concentration (mg F^-/L) gave equations typical of second order, enzyme-mediated reactions. Brown trout from a natural population exposed to as much as 14 mg F^-/L showed a linear relationship between bone concentration and body length (hence, age), suggesting a relatively continuous retention of fluoride over time. Fluoride in skeletal bone reached higher levels (>1,500 μg F^-/g) than cancellous bone (approximately 1,000 μg F^-/g) for rainbow trout and they accumulated fluoride faster than did the pollution-tolerant carp. The increase in mucous cells in the gills at low fluoride concentrations and in the skin of the head at high levels suggest that rainbow trout make some attempt to regulate fluoride using their chloride-secreting mechanism. Trout eggs showed an uptake rate pattern similar to that of bone. Gordon and Tourangeau (1977) reported preferential accumulation of fluoride in the bone (1,351 μg F^-/g) of three trout species in a previously contaminated stream; skin contained 146 μg F^-/g and muscle only 8.7 μg F^-/g. A similar pattern was reported for tilapia fish (*Oreochromis leucostictus*) living in water containing 2.4 mg F^-/L. Only the bone concentration showed a relationship with fish weight, suggesting that the soft tissues have a saturation level or a fluoride excretion mechanism (Gikunju 1992). Kudo and Garrec (1983) reported accumulation factors of 9 to 32 times in carp exposed to ammonium fluoride in an experimental pond.

Christensen's (1987) comparison of a river-dwelling population of Arctic char (*Salvelinus alpinus*) exposed to from 5 to 20 mg F^-/L to a nearby anadromous population showed that, like invertebrates, this species is capable of releasing accumulated fluoride. It is possible that the physiological changes involved in moving from fresh to salt water are responsible for mobilizing the fluoride. As with trout, bone held higher concentrations (1,150 μg F^-/g) than muscle (16 μg F^-/g). He cited a study that indicated uptake in fish can occur from food as well as from water. Note that fish actually ingest very little water, so uptake from water must be primarily across the gills during respiration. Rainbow trout have been shown to accumulate ingested fluoride in their skeletal tissues, tolerating as much as 2,250 μg F^-/g sodium fluoride in their feed. Fluoride is readily available to salmonids fed krill and other marine organisms (Landy et al. 1991).

Wright (1977) showed that fluoride uptake in brown trout fry (*Salmo trutta*) tended to reach a plateau after 8 days, even at 20 mg F^-/L. The concentration factor at 5 mg F^-/L was 2.3. Pillai and Mane (1985) demonstrated that the fry of *Catla catla*, a tropical fish, accumulate fluoride from an effluent in a fashion directly proportional to effluent concentration and length of exposure. *Catla catla* eggs have also been shown to accumulate fluoride (Pillai and Mane 1984).

In the marine environment, Hemens and Warwick (1972) showed

that the mullet (*Mugil cephalus*) accumulates significant (55X) fluoride at 52 mg F^-/L. This accumulation factor is much higher than those of the accompanying invertebrates in their experimental ecosystem and suggests that the omnivorous mullet may have gained some of its fluoride from its food. Wright and Davison (1975) found that the axial skeleton concentrated far more fluoride (20 to 100 μg F^-/g [wet weight]) than any of the other tissues in a variety of fish species, but the data were too variable to show differences between fish caught near the contaminant source and those caught in the open ocean. Fish skin also concentrated fluoride to some degree (10 to 50 μg F^-/g [wet weight]). High fluoride content in food (mainly crustaceans) from stomachs of some cod (*Gadus morrhua*) contributed to elevated levels (20 μg F^-/g [wet weight]) in their stomach walls. One haddock (*Gadus aeglifinus*) from near the source had an extremely high level in its gill bar (100 μg F^-/g [wet weight]) and skin (75 μg F^-/g [wet weight]). Mudskipper (*Boleophthalmus dussumieri*) accumulated very little fluoride in intestine, muscle and liver during the first 48 hours of exposure to 5 mg F^-/L, but continued exposure lead to levels as high as the 50 mg F^-/L animals. After 10 days, however, the fluoride levels at all exposures had dropped, suggesting that the soft tissues of this species have the ability to release fluoride. Whether it was sequestered in the skeleton or eliminated entirely from the body was not determined (Shaikh 1986).

Key points: (i) Fish may accumulate higher levels of fluoride than invertebrates, from food as well as water. (ii) Fluoride in fish is generally sequestered in the skeleton and skin.

Mammals

The only information pertaining to fluoride in aquatic mammals refers to ocean-going species, i.e., fin whales and crabeater seals, both of which prey on krill. Extremely high levels of fluoride have been reported in the bones of these animals (3,070–18,570 μg F^-/g) without any apparent negative effect (Landy et al. 1991). Terrestrial mammalian species have been studied extensively. Fluoride ion appears to be absorbed fairly rapidly (half-life in stomach 30 minutes) and efficiently (85% plateau at 2 hours) by the mammalian digestive tract, with the kinetics of a typical first-order reaction (Smith 1966b). Whitford and Pashley (1984) presented evidence that absorption was principally as the undissociated molecule HF, not the fluoride ion itself, at the normal stomach pH of 2.1. Some absorption also occurs from the small intestine, where it has been suggested that the hydrated fluoride ion can successfully pass through paracellular channels (Nopakun and Messer 1990). The presence of calcium or aluminum ions reduces gastrointestinal absorption, presumably by forming insoluble complexes that do not easily cross membranes (Hodge and Smith 1965).

When uptake rates into blood are examined, efficiency is in the order of 2% per minute, which is low when considering water passing over gills as the major absorption route in fish. Soft tissue content generally reflects the blood concentration, equilibrating in 15 to 60 minutes, depending on the

blood supply to the tissue. Normal tissue levels range from 0.1 to 1 μg F^-/g and tissue accumulation is seldom greater than threefold except in extenuating circumstances such as kidney malfunction (Smith 1966b).

The majority (90%+) of absorbed fluoride is sequestered in mammalian skeletons. Kay et al. (1975) reported femur levels of 148.7 μg F^-/g in beaver and 266.4 μg F^-/g in muskrat, even in uncontaminated environments. Primary deposition involves exchange with hydroxyl ions on the surface of the well-defined crystalline hydroxyapatite ($Ca_{10}(OH)_2(PO_4)_6$) lattice, as OH^- and F^- are approximately the same size. At high exposure levels, phosphate may also be displaced. The same mechanisms are assumed to occur in fish, although in invertebrates the amorphous structure of the exoskeleton suggests simple deposition as CaF_2 (Wright and Davison, 1975). Skeletal deposition does seem to have a saturation level in mammals, at 15,000 to 20,000 μg F^-/g. Beyond this point, almost 100% is excreted in the urine. When exposure ceases, skeletal mobilization shows a half-life of 2 or more years. Excretion rates reflect intake and show first-order kinetics over time, although this is the culmination of a complex series of processes. Fluoride is filtered out in the glomeruli much like chloride, but is resorbed less efficiently. Faecal excretion is generally less than 10% and reflects the amount of insoluble fluoride ingested, although some re-release from the blood into the stomach does occur that contributes to the faeces (Hodge and Smith 1965).

Key point: Aquatic mammals accumulate fluoride in their skeletons and teeth and release it via the urine.

Birds

Birds are also capable of accumulating fluoride in their bones. Pelicans from a contaminated estuarine environment had 3,495 μg F^-/g (cited in Walton 1986). Carnivorous and omnivorous species have higher bone levels than herbivores, even in nonpolluted environments, suggesting biomagnification (Rose and Marier 1977). Black-crowned night herons living adjacent to a phosphate processing plant showed increasing skeletal fluoride concentrations with age, up to 6,042 μg F^-/g ash weight and a correlated increase in tibiae diameter and decrease in wall thickness, suggesting excessive internal bone resorption (Henny and Burke 1990).

Four Antarctic penguin species which consume a krill diet of more than 200 mg fluoride per day showed femur concentrations in the order of 10,000 μg F^-/g dry mass and soft tissue concentrations of 2 to 10 μg F^-/g dry mass. These penguins excreted most of the fluoride consumed. What was absorbed was effectively buffered by the skeleton, where it had a half-life of only 3 to 4 weeks (Culik and Adelung 1988; Machoy and Machoy-Mokrzyńska 1990).

Key point: Birds also accumulate fluoride in bones.

Biotransformation

A variety of food plants, forage crops and soil microorganisms are

capable of incorporating air-borne fluoride into organic acids (Groth 1975). Amounts formed have been shown to be related to the ability of the plant to extract fluoride from the environment. Fluoroacetate is the most common compound formed (Miller et al. 1973). Fluoroacetate combines with oxaloacetic acid in the tricarboxylic acid cycle, an integral part of respiration in all organisms (Ward and Huskisson 1972). The fluorocitrate thus formed binds irreversibly with aconitase, the enzyme that normally dehydrates citric acid to cis-aconitic acid. Citric acid then accumulates, leading to hypocalcemia and eventually heart failure in animals (Amdur et al. 1991). Fluoroacetate has been used to exterminate insects and rabbits (Albert 1979) and is 15 times more toxic to humans than is sodium fluoride (Matsumura 1975). No information is available on fluoroacetate formation in aquatic microorganisms, algae or plants, but their low ability to absorb fluoride suggests that even if they are capable of the synthesis, insufficient quantities would be formed to be harmful to the animals that consume them.

Key point: There is no evidence of significant biotransformation processes in aquatic systems.

Toxicity

Lethal Threshold

Terrestrial systems

Inorganic fluoride compounds such as sodium fluoride (NaF), cryolite (Na_3AlF_6) and sodium fluorosilicate (Na_2SiF_6) have been used since the beginning of the century as insecticides, and cases of human poisoning from mistaken use of "roach powder" as a baking ingredient have occurred consistently throughout the years (Hodge and Smith 1965). The oral LD_{50} for humans is estimated at 75 mg/kg for sodium fluoride and 125 mg/kg for sodium fluorosilicate (Matsumura 1975). For mammals in general, the oral LD_{50}'s range from 20 to 100 mg/kg or a blood concentration of 8 to 10 ppm (WHO 1985). On the relative scale presented in Amdur et al. (1991), this puts fluoride compounds in the "very" to "extremely" toxic range and equivalent to DDT in acute toxicity. Symptoms of acute poisoning in humans include nausea, vomiting and diarrhoea, muscle and heart fibrillation, spasm and some paralysis, respiratory and cardiac depression, leading to coma before death. Internal examination reveals extensive congestion and mucus accumulation in digestive and respiratory tracts and damage to mucous membranes, particularly the intestinal epithelium. Basically, the normal metabolism of the cells is blocked and cell membrane permeability altered (SBSC 1979). Symptoms of toxicity in field voles included a rapid fall in body weight, loss of physical condition, diarrhoea, excessive thirst and the presence of severe dental abnormalities (Boulton et al. 1994). The specific cause of death has not been identified, but may involve enzyme inhibitions, calcium complex

formation, dehydration and electrolyte loss, specific organ injury, particularly in the kidney and nervous system, or a combination of all the noted physiological effects (Hodge and Smith 1965).

Free-living protozoa and rotifers are fairly resistant to fluoride intoxication, no acute toxicity being observed before 1,000 mg F^-/L. Some decrease in movement and occasional cyst formation was observed in concentrations as low as 2 mg F^-/L, however (Wantland 1956). Microorganisms are also generally resistant to fluoride. Clarkson et al. (1989) showed no effect on nitrification of a concentrated industrial waste below 200 mg F^-/L, then increasing inhibition to a plateau level at 800 mg F^-/L. Yeasts during fermentation are sensitive to concentrations of 30 mg F^-/L (Hodge and Smith 1965). Van Wensem and Adema (1991) demonstrated toxicity to soil microorganisms, as measured by the rate of nitrification in poplar litter, above 0.1 mg F^-/g dry weight

Key points: (i) Fluoride falls in the "very" to "extremely" toxic range for terrestrial systems. (ii) Specific cause of death in acute toxicity is not clear. (iii) Microorganisms and protozoa are relatively resistant to fluoride poisoning.

Aquatic systems

Aquatic studies generally indicate a low degree of acute toxicity for fluoride. In the process of setting water quality standards for North Carolina, an extensive review of fluoride toxicity in fresh water was conducted (WQPB 1986). The studies were performed under widely varying conditions of pH, temperature, hardness and duration of exposure. Lethal levels (generally LC_{50}'s) reported range from 2.7 mg F^-/L for rainbow trout (the most sensitive species) and 98 mg F^-/L for *Daphnia magna* to 900 mg F^-/L for frogs, and 123 to 1,900 mg F^-/L for algae and aquatic plants. Data for rainbow and brown trout have been extracted from the report and are presented graphically in Fig. 2. An incipient lethal level (the concentration at which toxic effects may be expected over very long periods of exposure) has been determined by these authors as roughly 2.5 mg F^-/L.

More recent studies generally support the conclusions of the North Carolina report. Samecka-Cyerman and Kempers (1990) found a 48 hour LC_{100} of 400-450 mg F^-/L for aquatic liverworts (*Scapania undulata*), but reported that 100 mg F^-/L for 16 days was harmless. Mane and Gokhale (1990a) demonstrated LC_{50}'s between 430 mg F^-/L and 600 mg F^-/L for the bivalve *Lamellidens marginalis*, depending on the season. There was a less-than-twofold difference between LC_0 and LC_{50} levels in this study. Five cladoceran invertebrates had LC_{50}'s from 83.2 mg F^-/L to 353.6 mg F^-/L (Hickey 1989). The aquatic larvae of five Spanish net-spinning caddisflies (Trichoptera) were more sensitive to fluoride, having 96 hour LC_{50}'s of 26.3 to 44.9 mg F^-/L (Camargo and Tarazona 1990; Camargo et al. 1992a). Three American caddifly species had 96 hour LC_{50}'s ranging from 17.0 to 42.5 mg F^-/L (Camargo et al. 1992b). Time-related toxicity data for the two most sensitive caddisfly species have been extracted from those reports and are presented graphically in Fig. 3. Fingernail clams (*Musculium trans-*

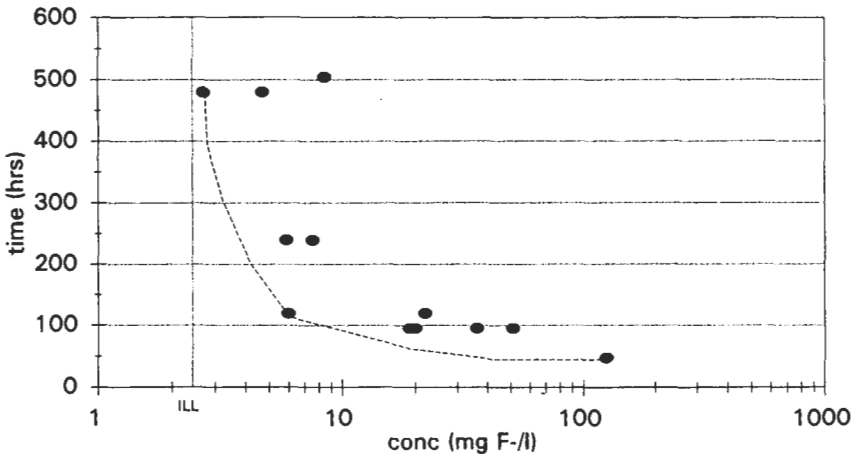

Fig. 2. The distribution of time-related lethal toxicity data for trout species, showing the apparent incipient lethal level (ILL). Data taken from WQPB (1986).

versum) are particularly sensitive, a 50% mortality rate being reported at a concentration of 2.8 mg F^-/L in an 8-week flowthrough experiment (Government of Canada 1993).

The final acute value calculated for North American species and North Carolina water conditions in the WQPB study was 15.6 mg F^-/L,

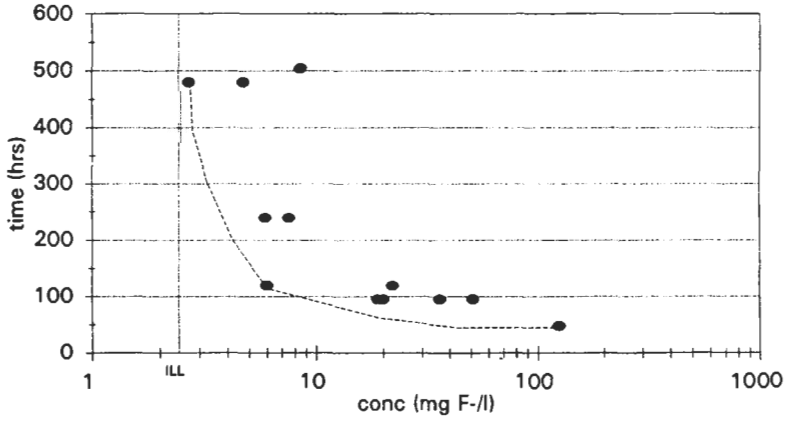

Fig. 3. The range of time-dependent EC50 and LC50 values and a calculated NOEC level for two sensitive species of Hydropsyche (caddisfly) aquatic larvae. Data are from Camargo et al. (1992a and b).

roughly half the value used by the EPA when setting water quality standards in 1977. Camargo and Tarazona (1991) calculated a maximum acceptable toxic concentration (MATC) of 27.6 mg F^-/L in soft water for rainbow trout. In general, acute fluoride toxicity increases with increasing temperature (Angelovic et al. 1961) and decreasing hardness (Pimental and Bulkley 1983). Given the relatively low temperatures and high hardness of the St. Lawrence River in the present study zone, lethal toxic effects due to fluoride would not be expected to occur even at the mouth of the treatment plant outlet. However, these "safe" values should be compared with those for chlorine, which was initially reported to be toxic to fish in the range of 100 to 1,000 mg/L, but when chemical interactions with other materials in water were taken into account, the residual chlorine was shown to be acutely toxic at levels below 1 mg/L. Total residual chlorine now has a recommended "likely long-term safe level" of 0.002 mg/L for continuous release into receiving fresh waters (Brungs 1973; Hellawell 1988). A similar change in "target" speciation may eventually occur for fluoride, particularly since Nichol et al. (1987) demonstrated that toxic effects on algae varied directly with the concentration of the hydrogen fluoride molecule, starting at 0.04 mg/L.

Symptoms of fluoride poisoning in fish are apathy and loss of appetite, followed by violent sporadic movement, loss of equilibrium, muscle tremors and prolonged contraction, high mucous secretion and finally death (Neuhold and Sigler 1960; WHO 1985). These effects are sufficiently similar to those of mammals, birds (Andreasen and Stroud 1987) and insects to suggest a related toxic mechanism for all species.

Key points: (i) Fluoride ion is not very toxic to aquatic systems. (ii) The most sensitive aquatic organisms are certain pelagic fish species. (iii) Acute fluoride toxicity is greatest in warm, soft water.

Sublethal Threshold

Terrestrial systems

Effects of low doses of and chronic exposure to fluorides have been well studied in terrestrial animals (i.e., Suttie 1977). The major physiologic action is the alteration in dental and skeletal structure caused by the formation of fluoroapatite. Brittle, discoloured teeth, osteoporosis, lameness and postural changes result. Stiffness in the joints due to calcification of the tendons occurs. Loss of appetite, decreased mobility and difficulty chewing lead to weight loss and reduced productivity (WHO 1985; Boulton et al. 1994). Monsour and Kruger (1985) reviewed fluoride intoxication effects on soft tissues in vertebrates, noting the enigmatic nature of its actions and lack of specificity in tissues damaged and normal functions disrupted. The diagnosis of fluorosis cannot be made on any one symptom, but requires the correlation of urine, bone and tissue analyses, skeletal X-rays, biopsies and necropsies, and determination of fluoride levels in food consumed (SBSC 1979).

Plants exposed to low levels of fluoride exhibit chlorosis (yellowing)

of leaves or needles, followed by tissue necrosis or leaf wilting. Eastern white pine (*Pinus strobus*) also showed reduced transpiration rates and changes in membrane permeability (Rakowski and Zwiazek 1992). Sensitive species may show reduced yield or decreased seed production, and some species experience altered growth rates, including increased but less healthy biomass production (Weinstein 1977; Canada 1993).

Key points: **(i)** Fluoride alters dental and skeletal structure, resulting in deformation and brittleness. (ii) Changes in membrane permeability are the initial effects of chronic fluoride exposure in plants.

Aquatic systems

When data gathered in the North Carolina report for sublethal effects and "no-observed-effect" concentrations (NOEC) are compared to those causing lethality, there does not appear to be a very significant difference. For example, the ratio of growth NOEC to LC_{50} for *Daphnia magna* is 0.11 (Fieser et al. 1986). Chronic:acute ratios calculated from data in the report range from 1.49 to 0.07, and they recommend that an average value of 0.25 might be more accurate than the 0.05 rate used originally by the U.S.EPA, pending more appropriate and extensive chronic studies. Samecka-Cymerman and Kempers (1990) reported a chronic:acute ratio of 0.25 for aquatic liverworts. Hickey (1989) reported chronic:acute ratios of 0.14 and 0.17 for two *Daphnia* species. The ratio value of 0.05 is normally applied when insufficient chronic data exist to indicate otherwise and when bioaccumulation in an available form for food chain transfer does not occur to any significant degree. There remains a need for more careful study of chronic exposure effects in order to resolve the ratio issue, particularly as there is an indication that fluoride bioaccumulation occurs.

Sublethal toxicity can be difficult to quantify since the impact may only be observable at the population or community level. At the individual level generally studied in the laboratory, subtle changes in physiological parameters may go unnoticed or their significance unrecognized (Groth 1975). Nichol et al. (1987) suggested that a reduction in cellular ATP levels was responsible for the growth lag and inhibition of photosynthesis noted in cyanophyte algae exposed to between 5 and 150 mg F^-/L. The algae did eventually recover, however, and an ability to develop resistance to higher levels of fluoride was demonstrated, making the long-term effects of low-level fluoride exposure yet more difficult to discern.

Studies have been done on growth and reproductive effects of fluorides on *Daphnia magna* indicating an NOEC of 5.2 mg F^-/L for standard test methods, but when the data were examined from a slightly different perspective, subtle reproductive effects were evident at concentrations as low as 0.4 mg F^-/L (Dave 1984). The Indian bivalve *Lamellidens marginalis* exposed to non-lethal as well as lethal concentrations of fluoride showed significant changes in glycogen, protein and lipid contents in various body parts that reversed normal seasonal biochemical changes

(Mane and Gokhale 1990b). Reddy and Venugopal (1990a, 1990b) have shown effects on protein metabolism, acetylcholinesterase activity and oxygen consumption of the Indian freshwater field crab *Barytelphusa guerini* at a sublethal fluoride level of 30 mg F^-/L. Equivalent work seems to be lacking for North American freshwater species, although Damkaer and Dey (1989) demonstrated an important impact of fluoride on Pacific salmon migratory patterns above 0.2 mg F^-/L. This is the concentration recommended by Foulkes and Anderson (1994) as the "criteria level" in fresh water to protect salmon species in the Pacific Northwest.

Ecotoxicological studies done on a Spanish river indicated that the release of a fluoride-containing industrial effluent contributed to only a minor degree to the elimination of rainbow and brown trout populations (Camargo 1991a) and alteration of caddisfly assemblages (Camargo 1991b) and macroinvertebrate communities (Camargo 1992) downstream. Levels of fluoride in the river were well below acute toxic levels, but above the calculated sublethal NOEC of 2.51 mg F^-/L for sensitive caddisfly larvae (Camargo et al. 1992a) for 2.5 km below the outlet. A similar study in Colorado for a municipal wastewater fluoride source contributing to a level of 1.17 mg F^-/L in the river also concluded that fluoride pollution was not a major factor in determining the spatial distribution and abundance of caddifly larvae (Camargo et al. 1992b). It is interesting to note that the sublethal EC_{50} values for the caddisfly larvae determined by Camargo et al. (1992a) fall in the same range as the lethal LC_{50} values presented in Fig. 3. This would suggest that the critical endpoint to examine in terms of risk is the death of organisms, as there is little indication that sublethal and chronic effects are occuring at significantly lower concentrations.

Key points: (i) There is rarely a large difference between acute and chronic (or lethal and sublethal) threshold levels of fluoride in aquatic systems. (ii) There is some evidence to suggest that low levels of fluoride do not significantly alter aquatic community structures.(iii) Some species may exhibit avoidance behaviors that impact on reproductive success.

Toxic Mechanisms

Enzymes and physiological processes

Fluoride is a well-known enzyme inhibitor, often used to study enzyme action and structure, without relating the observed action to fluoride toxicity in whole organisms. Enzyme inhibition often has implications for survival and reproductive success at levels far below the lethal threshold. The capacity of fluoride to alter enzyme structure contributes to the concern about fluoride in the environment. As noted in the previous section, this may not be the case for fluoride in aquatic systems, but an examination of the subject is justified nonetheless. The interactions may involve HF and HFF^- as well as F^-, depending on the ambient pH employed in the experiment or expected in the tissue (Wiseman 1970). Emsley et al. (1981) pointed out the significance in biological systems of

fluoride's ability to form strong hydrogen bonds with amides. Protein-based compounds such as enzymes are dependent for their function on complex tertiary and quaternary structure maintained by hydrogen bonds between component amino acids and between amino groups and water molecules. For example, Edwards et al. (1984) demonstrated subtle alterations in the crystalline structure of yeast cytochrome c peroxidase, due to the bonding of HF (at 20 mg F^-/L) to the iron at the heart of the enzyme, replacing a water molecule. The active site was disrupted by the attraction of two amino groups and the addition of a new water molecule, changing positions just enough to prevent the enzyme from bonding with cytochrome c. Wiseman (1970) presented a review of fluoride inhibitory action, primarily in mammalian cells and tissue extractions and homogenates, dividing the enzymes affected into four main groups: (1) requiring metal ions and enhanced by phosphate, i.e., enolase, phosphoglucomutase, lecithinase, succinic dehydrogenase; (2) requiring divalent metal ions, i.e., acid and alkaline phosphatases, pyrophosphatases, kinases, cholinesterase, glutamine synthetase, alcohol dehydrogenase; (3) no metal ions required, i.e., liver esterase or lipase, 5'-adenylic acid deaminase, phosphoglycerate mutase, hydrazidase; and (4) containing trivalent metal ions, i.e., catalase, peroxidase.

Additionally, Olson and Christensen (1982) report that levels of fluoride just above 1 mg F^-/L inhibit the activity of urease, an enzyme common in plants, algae, bacteria and microorganisms. Wilke (1987) reported decreases in the activity of dehydrogenases, alkaline phosphatases and aryl sulphatases in soil microorganisms exposed to low fluoride levels.

Although inhibition is much more commonly observed, fluorides may also activate enzymes. It is thought that this effect may be due to interference with the formation of larger carbohydrates, thus making more of the small substrate molecules available for metabolism. This situation induces higher activity of the appropriate enzymes (Hodge and Smith 1965). Also, when glycolysis is blocked by fluoride, the enzymes of the hexose monophosphate shunt are stimulated (Saralakumari and Ramakrishna 1991). The effects of fluoride on bone structure involve stimulation of osteoblast precursors to increase phosphate transfer across the plasma membrane, either by causing the synthesis of new P_i carriers or speeding the turnover of existing ones (Selz et al. 1991).

Examination of fluoride effects on enzymes in aquatic organisms are rare, but two papers should be noted. Christensen (1971) found no effect on in vitro glutamic oxalacetic transaminase and lactic dehydrogenase from white sucker (*Catostomus commersoni*) blood plasma even at 2,000 mg F^-/L, although heavy metal cations with known aquatic toxicity showed effects at 50 mg F^-/L. Neither of these metabolic enzymes is known to have a metal cofactor as so many of the enzymes affected by fluoride do. Carbonic anhydrase, an enzyme that catalyses the transport of carbon dioxide from tissues to red blood cells and hence to water in the gills of channel catfish (*Ictalurus punctatus*), showed no effect below 20 mg F^-/L in vitro (Christensen and Tucker 1976).

Matthews (1970) attempted to put all these enzyme interactions into perspective by discussing changes in cell and tissue function as a consequence of fluoride intoxication. Metabolic pathways, physical activity, cell growth and cell development are all affected at some point. Adenosine triphosphate (ATP) stores the energy obtained from food and is essential for all energy-consuming cell activities. Without ATP, there is no life (Blaise et al. 1986).

The pathways affected by fluoride include (1) energy production by glycolysis, (2) pyruvate metabolism and oxidation, (3) oxidative phosphorylation, (4) respiration, (5) protein synthesis, (6) hormonal response, and (7) fat metabolism. Physical activity includes sperm motility, phagocytosis, contractility and transport across cell membranes. Ion balance in cells can be disrupted by inhibition of the sodium, potassium and calcium-dependent adenylate triphosphatases known as cation transport ATPases (i.e., Murphy and Hoover 1992). Coll and Murphy (1992) showed that the tight binding of two fluorides and one magnesium ion to the phosphorylation site of calcium ATPase blocks the transfer process. Calcium balance destroyed either through enzyme inhibition or direct precipitation of CaF_2 can affect nerve transmission and blood coagulation and further disrupt cell permeability (SBSC 1979).

Key point: Fluoride is an effective enzyme inhibitor acting on a wide variety of internal systems.

Mutagenicity

Much has been made of the possible link between fluoride and cancer (i.e., Bundock et al. 1985). Studies indicating chromatid damage and chromosome abnormalities in vitro abound but are contradicted by others. Definite evidence has been presented regarding unscheduled DNA synthesis at exposure levels below 1 mg F^-/L that support the concern regarding mutagenicity (Zeiger et al. 1993). Little extension to in vivo work seems to have occurred, however, particularly for aquatic species. Additionally, the fluoride levels used for many of the in vitro works were higher than those likely to be encountered in tissues of aquatic organisms living in water with a fluoride concentration of less than 1 mg F^-/L.

In vitro inhibition of DNA repair mechanisms has been reported by some researchers at levels of 0.4 to 1.0 mg F^-/L (SBSC 1979; WHO 1985). Although their direct carcinogenic action is questionable, the potential is present for fluorides to act as cofactors in the presence of known mutagens in that damage done by the other compounds would not be as readily corrected. More research is required to define the degree of potentiation that fluorides may be capable of producing. An extensive review of fluoride potential for genetic toxicity (Zeiger et al. 1993) concluded that the issue of chromosome damage in vivo was unresolved and that research was needed into possible mechanisms for observed effects, including the extent of F^- interaction with DNA-associated proteins or enzymes.

Key point: Fluoride has not been clearly shown as directly carcino-

genic but may be a cofactor.

Synergisms

Aluminium

As mentioned in the section on transport, the mobilization of aluminium in the presence of low concentrations of fluoride is a matter of environmental concern (Driscoll et al. 1980). Formation of fluoro-aluminium complexes does lower the toxicity of aluminium to juvenile Atlantic salmon (*Salmo salar*), but the complexes retain some toxic action (Wilkinson et al. 1990). The toxic mechanism of $Al(F)_x$, like that of aluminium alone, involves the upset of osmoregulation and the reduction in plasma sodium concentration, even at sublethal levels of 1.0 μM/L. Parkhurst et al. (1990) showed no difference in aluminium toxicity to brook trout fry (*Salvelinus fontinalis*), despite an excess of fluoride ion available for complex formation with the aluminium. Work has generally been conducted in soft water at relatively low pH, however, simulating conditions in the Canadian Shield, rather than the moderately hard, slightly alkaline conditions existing in the St. Lawrence River. The potential for ecological damage by fluoro-aluminium complexes remains to be confirmed in the receiving waters of the Montréal sewage treatment plant.

Of note regarding human consumption of fluoride is its ability to release aluminium from cooking utensils (Tennakone et al. 1988), notably in the presence of boiling fruit acids (Walton 1989). Once ingested and absorbed into the body, aluminium causes altered calcium metabolism in several tissues, including the brain, and is implicated in a number of neurological disorders, notably Alzheimer's disease (Amdur et al. 1991). Wei et al. (1995) suggested a combined toxicosis between fluoride and aluminium based on experiments with chickens, rabbits and rats, in an attempt to explain unusual symptoms observed in some human patients from a severe fluorosis region.

Copper

Research on the green algae *Chlorella vulgaris* has shown a cessation of respiration on joint application of fluoride and copper at concentrations that have no effect when applied separately (Hassall 1969; Sargent and Taylor 1972). Levels of both copper and fluoride were, however, 10 to 100 times greater than those existing in the St. Lawrence River (Sloterdijk 1988). It does not seem likely that the algal community that forms the base of the aquatic food chain is threatened by this synergism.

Chloride

Neuhold and Sigler (1960) cite a study indicating that fluoride toxicity increases for the mosquitofish (*Gambusia affinis*) in the presence of chlorine. This is in agreement with studies of halogen interactions on

Chorella algae by Kott and Edlis (1969). However, work on rainbow trout indicates that chloride tempering decreases the response to a given concentration of fluoride, presumably due to the activation of chloride-secreting cells in the gills (Neuhold and Sigler 1962). Chloride levels of 3 to 48 mg/L (Sloterdijk 1988) in the St. Lawrence River may thus have a protective effect with respect to additional fluoride from the sewage outlet.

Organic Compounds

Studies on the effect of fluoride on the liver microsomal cytochrome-P450 system that is often implicated in the metabolism of organic compounds are contradictory. Post and Snyder (1983) found that benzene metabolism was stimulated by fluoride concentrations greater than 10 mM/L. Do Phuoc et al. (1983) found that inhaled fluoride at 4 ppm inhibited total cytochrome-P450 levels in rat liver, but induced dimethylnitrosamine-demethylase, thus increasing the cancerogenic potential of DMN. Bompart et al. (1988) reported no effect on benzo(a)pyrene metabolism due to inhaled HF. The metabolism of organic contaminants is a complex subject that requires further in-depth research before any conclusions can be drawn regarding possible synergisms with fluorides, but it appears that there is some potential for synergism with certain kinds of organic pollutants.

Miscellaneous

Marier (1972) and Weinstein (1977) note that the presence of boron in soil or applied fertilizers increases the uptake of fluoride by plants. The extent of this particular interaction is difficult to assess in the current situation, as boron content is not normally determined in Quebec waters and the applicability of the observed effect to aquatic plants is unknown.

Del Razo et al. (1993) noted the correlation between high arsenic and high fluoride concentrations in drinking water in an area of Mexico and suggested that some of the symptoms attributed to arsenic poisoning may be due to fluoride. Both elements affect enzyme activity in the glycolytic and tricarboxylic acid pathways, depress succinate dehydrogenase activity and disrupt heme synthesis.

The Alcan Surveillance Committee (ASC 1979) felt that there was a link between fluoride stress and susceptibility to insect infestation in trees near the Alcan smelter at Kitimat, B.C. This interaction is of the subtle type that is difficult to confirm in the laboratory and subject to a variety of interpretations when observed in the field. Similarly, combined effects of sulfur dioxide and fluoride air pollution have been reported in the field (Murray and Wilson 1988). Implications for aquatic systems are impossible to determine. Such occurrences are, however, consistent with the overall assumption that any new and foreign contaminant that an organism must deal with reduces its viability and reproductive capacity.

Key point: The only significant evidence of synergistic action is with aluminium, in which the increased mobilization of aluminium may be balanced by the decreased toxicity of fluoro-aluminium complexes.

Summary

Water fluoridation by the city of Montréal would add only a small amount of fluoride to the St. Lawrence River as a whole, although it may be enough to push some kinds of organisms over sublethal thresholds in the immediate receiving waters. The small difference between no-effect and lethal levels of fluoride in aquatic systems is significant; lethality would appear to be the critical endpoint for risk assessment purposes. The physicochemical conditions of the river reduce the potential for lethal toxic action considerably. The existing community is relatively pollution-tolerant, and there are no behaviorally sensitive species like the Pacific salmon present that would be affected in the long term. Therefore, it is concluded that the fluoridation of the city of Montréal's drinking water would pose only a minimal risk to the aquatic community in the immediate receiving waters of the St. Lawrence River, due to a minor additional stress to enzyme systems within the few organisms present. Should the city decide to fluoridate, a biomonitoring program is recommended, measuring the activities of a few target enzymes in the benthic species present.

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