BIOMARKERS OF STRESS IN URBAN RIVERS: MIXED-FUNCTION-OXYGENASE AND ACETYLCHOLINESTERASE EFFECTS IN BROWN TROUT IN RIVERS IN ST. JOHN'S, NEWFOUNDLAND

J. F. PAYNE[1], W. MELVIN[2], A. MATHIEU[1] AND L. FANCEY[1]

[1]Science Branch
Department of Fisheries and Oceans
P.O. Box 5667
St. John's, Newfoundland A1C 5X1

[2]Oceans Limited
31 Temperance Street
St. John's, Newfoundland

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J. F. Payne[1], W. Melvin[2], A. Mathieu[1] and L. Fancey[1]

Science Branch
P.O. Box 5667
St. John's, Newfoundland
A1C 5X1

[1]Science Branch, P.O. Box 5667, St. John's, Newfoundland A1C 5X1
[2]Oceans Ltd., 31 Temperance Street, St. John's, Newfoundland

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ABSTRACT

Payne, J. F., W. Melvin, A. Mathieu and L. Fancey. 1994. Biomarkers of Stress in Urban Rivers: Mixed-function-oxygenase and acetylcholinesterase effects in brown trout in rivers in St. John's, Newfoundland. Can. Tech. Rept. Fish. Aquati. Sci. No. 1947: v + 23 p.

The pollution of urbanized lakes and streams has traditionally been viewed as an acceptable trade-off of urban life. However growing environmental sensitivities have focused attention on the social and economic value of such waterways and there are increasing calls for water quality improvement. Biological markers are sensitive, cost effective tools for evaluating the risks of environmental contaminants. Two markers of contaminant stress, namely mixed function oxygenase enzyme induction, which has gained wide acceptance in environmental studies over the past few years, as well as acetylcholinesterase enzyme inhibition, an indicator for neurotoxicity, were evaluated in two rivers, Virginia River and Rennies Mill River, for which the City of St. John's is a major watershed area. Marked differences in enzyme activity were observed in brown trout in the two City Rivers in comparison with a reference river, South Brook, which is located just outside the City. Predictions can not be made about possible population level impacts but it is reasonable to state that water quality in St. John's rivers is impaired to a sufficient degree to produce early warning symptoms of contaminant stress in fish. Accordingly, any additional loadings would be expected to exacerbate the situation. With respect to water quality improvement, the biomarkers used in the study will be valuable for assessing any future remedial measures. Also, given the importance of acetylcholinesterase as a potential biomarker for neurotoxicity, the observations on this enzyme in St. John's rivers will be of value for international interests involved with the development of biological monitoring and assessment indices.

RESUME

Payne, J. F., W. Melvin, A. Mathieu and L. Fancey. 1994. Biomarkers of Stress in Urban Rivers: Mixed-function-oxygenase and acetylcholinesterase effects in brown trout in rivers in St. John's, Newfoundland. Can. Tech. Rept. Fish. Aquati. Sci. No. 1947: v + 23 p.

La pollution des lacs et des cours d'eau en zone urbanisée a depuis toujours été considéré comme une contrepartie acceptable de la vie urbaine. Cependant les sensibilités environementales grandissantes ont attiré l'attention sur l'importance sociale et économique du reseau hydrographique et on observe une demande sans cesse croissante de l'amélioration de la qualité des eaux. Les marqueurs biologiques sont des outils sensibles et d'un coût raisonnable permettant l'évaluation des risques entrainés par les contaminants environementaux. Deux marqueurs de stress, à savoir l'induction de l'enzyme oxygénase à fonction mixte qui a connu une large reconnaissance depuis ces dernières années ainsi que l'inhibition de l'enzyme acétylcholinestérase, un indicateur de neurotoxicité, ont été évalués dans deux rivières, Virginia River et Rennies Mill River dont St. John's est le bassin hydrographique. Des différences

notables ont été observées pour des truites brunes dans les deux rivières de la ville en comparaison d'une rivière référence, South Brook, située juste à l'extérieur de la ville. Si des prédictions quant à l'impact sur les niveaux de population ne sont pas possibles il est raisonnable d'affirmer que la qualité des eaux des rivières de St. John's a connu une dégradation suffisante pour entrainer l'apparition des premiers symptômes avertisseurs de stress dûs à la présence de contaminants dans le biotope des poissons. En conséquence tout ajout contribuerait à exacerber la situation. En ce qui concerne l'amélioration de la qualité des eaux, les biomarqueurs utilisés lors de cette étude permettraient de juger de l'impact de toute mesure d'amélioration prise. De plus, étant donné l'importance de l'acétylcholinestérase comme potentiel de biomarqueur de neurotoxicité, les observations faites sur cette enzyme dans les rivières de St. John's représentent une valeur dans le développement du suivi biologique et des indices d'évaluation.

INTRODUCTION

Problems in detecting impacts on marine organisms at population and community levels of biological organization has led to suggestions that changes in various physiological/biochemical parameters may be of value as early warning indices for identifying and delineating the sublethal effects of pollutants. The International Commission for the Exploration of the Seas convened a Workshop in Beaufort, North Carolina in 1979 to discuss this aspect of biological monitoring (McIntyre and Pearce, 1980). At this Workshop, a number of biochemical techniques were considered including the induction of MFO enzymes, for which a few field studies already existed including from sites in Newfoundland.

MFO enzymes play a critical role in detoxification by carrying out a series of oxidation reactions whereby relatively insoluble organic compounds are converted into water-soluble metabolites which may be further conjugated and excreted in urine or bile (see reviews by Bend and James, 1978; Lech et al. 1982; Payne, 1984). The term mixed-function refers to the fact that one atom of molecular oxygen is reduced to water while the other is incorporated into an enzyme substrate. Substrates include foreign compounds such as many drugs, pesticides and polycyclic aromatic hydrocarbons, as well as endogenous substrates such as steroid hormones, vitamins and bile acids. MFO enzymes have iron-containing heme-proteins, cytochromes P-450, as terminal oxidases and these oxidases are unique in that increased levels as well as variant forms are commonly found in the tissues of animals exposed to various types of inducing compounds. Potent inducers include drugs, selected natural compounds, and many xenobiotics of major environmental interest including polycyclic aromatic hydrocarbons (PAH) (Gerhart and Carlson, 1978), polychlorinated biphenyls (PCBs) (Addison et al., 1982), polybrominated biphenyls (PBBs) (Franklin et al., 1982), and petroleum hydrocarbons (Payne and Penrose, 1975; Chambers, 1979; Collodi et al., 1984).

The earliest field studies on the use of MFO enzymes as biological markers were carried out in the early 1970's (e.g. Payne and Penrose, 1975; Kurelec et al., 1977) and its use as a biomarker has now been confirmed in a large number of field studies worldwide (see recent reviews and references therein by Payne et al., 1987; Stegeman et al., 1992; Goksoyr and Forlin, 1992). Studies have been carried out in association with a variety of sources of contamination including urban runoff and industrial outfalls (e.g. Kezic et al., 1983; Fossi et al., 1986; Jimenez et al., 1990), pulp mills (e.g. Anderson et al., 1987; Munkittrick et al., 1991; Hodson et al., 1992) petroleum development sites (Davies et al., 1984) and agricultural spray programs (Vindimian et al. 1993). There have also been more broadscale studies including in the North Sea (Renton and Addison, 1992), the Great Lakes (Luxon et al., 1987) and the Mediterranean Sea (LaFaurie et al.; 1989; Narbonne et al.; 1991).

The high level of contamination associated with urbanized lakes and streams has traditionally been recognized as a "given". However, growing environmental sensitivities have focused attention on such situations and there are increasing calls for improvement. The prime purpose of this study was to determine if a case can be made for biologically important levels

of contaminants in St. John's rivers through an examination of MFO enzyme levels in fish.

In addition to MFO enzymes, another biomarker, namely the enzyme acetylcholinesterase (ACE), was also studied as an indicator for neurotoxicity. Acetylcholinesterase is involved in the deactivation of acetylcholine at nerve endings, preventing continuous nerve firings and as such is vital for normal functioning of sensory and neuromuscular systems (e.g. Murphy, 1986).

The mode of action of many organophosphate and carbamate pesticides is through inhibition of ACE, the enzyme systems of many invertebrate pests being particularly sensitive (e.g. Grue et al., 1991; Kennedy, 1991; Edwards and Fisher, 1991). The inhibition of ACE has been used in conjunction with agricultural and forestry spray programs to assess the nature and extent of exposures of humans and wildlife to pesticides (e.g. Thompson, 1991; Greig-Smith, 1991; Zinkl et al., 1991). However, a recent study in Europe has demonstrated that in addition to such point source studies, ACE could be greatly expanded as an environmental biomarker (Galgani et al, 1992). The study has attracted considerable attention since it produced evidence for variation in ACE in muscle tissues of fish along a pollution gradient in the North Sea.

The present study has produced evidence for differences in levels of both MFO and ACE enzymes in fish in two urban rivers in St. John's in comparison with a nearby reference river.

METHODS AND MATERIALS

HOMOGENATE PREPARATION

Approximately twenty-four brown trout of comparable size were collected using an electrofisher from each of three sites in and around St. John's. Site 1 was Virginia River; Site 2 (the control site) was South Brook and Site 3 was Rennie's River. Fish were killed by cervical dislocation and length and sex recorded. Entire fish were placed on dry ice and returned to NAFC where they were stored at -60°C for one month.

Brain and muscle tissue of individual trout were examined for ACE activity while liver was examined for MFO activity. Each fish was thawed slightly before excision of the whole brain. Approximately 1 g of muscle was removed from just below the dorsal fin on the right side and the entire liver was excised. Brain and muscle samples were homogenized in 0.05 M Trizma buffer, pH 8 at a ratio of 1 ml of buffer for each 100 mg of tissue. Liver was homogenized (1:4, w:v) in 40 mM Tris buffer, pH 7.5. Homogenates were prepared using ten passes of the pestle of a Ten Broeck hand tissue grinder. Muscle and liver fractions were centrifuged at 9,000 g for 10 minutes and the supernatant was removed and frozen at -80°C.

MIXED-FUNCTION OXYGENASE ACTIVITY - EROD

Ethoxyresorufin o-deethylase (EROD) activity was assayed fluorometrically as described by Porter et al. (1989) using a Perkin-Elmer LS-5 fluorescence spectrophotometer. The reaction mixture, final volume 1.25 ml, contained 53 nmol Tris-sucrose buffer (50mM, pH 7.5), 50 ul S9 liver, 2.25 nmol 7-ER (150 um ethoxyresorufin) and the reaction was started by the addition of 0.16 mg NADPH (1.25 mg/ml). After a 15-minute incubation at 27 C in a temperature controlled water bath, the reaction was terminated by the addition of 2.5 ml of ice-cold methanol. Methanol blanks contained the same components as the sample tubes with methanol being added to the addition of NADPH. Assay tubes were vortexed and the protein precipitate removed by centrifugation of 3600 x g for 5 minutes. The fluorescence of resorufin formed in the supernatants was measured in disposable acrylic cuvettes (1 cm path length) at 585 nm using an excitation wavelength of 550 nm (slit width of 0.5 mm). Enzyme activity was linear with time and protein concentration. The rate of enzyme activity in pmol/min/mg protein was obtained from the regression of fluorescence against standard concentrations of resorufin. Enzyme activity data were analyzed using ANOVA.

ACETYLCHOLINESTERASE ACTIVITY

ACE activity was assayed by the method of Ellman (1961) as modified by Hill and Fleming (1982) and Hill (1988). All assays were run in duplicate on a Perkin-Elmer recording, scanning spectrophotometer (Coleman 571). Acrylic cuvettes had a 1 cm optical path length and assays were conducted at 23°C. Absorbance was read at 405 nm.

The following was the analytical protocol used for the determination of ACE activity in this study (Fancey et al., 1990). In our experience there has been confusion surrounding the protocol for measuring ACE activity, thus our method is presented in detail. Enzyme activity data were analyzed using ANOVA.

A. Each sample cuvette received:

1. 3.0 mL of 2.5 x 10^{-4} M 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB), 661 mg Trizma HC1 and 97 mg Trizma base dissolved in 100 mL distilled water; pH 7.4. This solution is stable at 4°C in an amber bottle for 1-2mo.)

2. 100uL of 0.156 M acetylthiocholine iodide substrate (451.1 mg was made up to 10 mL with distilled water. This solution was prepared daily).

3. 20 uL of brain or muscle homogenate. Each homogenate was made at a ratio of 100 mg of tissue to 1 mL of 0.05 M Trizma buffer, pH.8 (4.4g Trizma HCl + 2.65 g Trizma base dissolved in 1000 mL of distilled water). Muscle tissue was centrifuged at 9,000 g and the supernatant kept.

B. For brain, the solution in the sample cuvette was mixed quickly using a pasteur pipette (~25 times), and placed in the spectrophotometer. For muscle, the solution in the sample cuvette was covered with parafilm and the cuvette was inverted 6 times. The reference cuvette received chromogen and substrate but no homogenate. The sample cuvette was placed in the spectrophotometer and the change in absorbance was recorded for 2 to 3 minutes at 405 nm, at 23°C.

C. The duplicate sample was assayed and the rate of change of absorbance per minute was determined from the chart recordings for both replicate samples.

D. Brain Enzyme Activity (umoles min^{-1} g^{-1})

Enzyme activity calculation (after Ellman et al. (1961), Hill (1980), and Fancey et al. (1990).

$$\frac{\Delta A/min \times Vol_t \times 1000}{E \times lightpath \times Vol_s \times tissue\ conc.} = \frac{\Delta A/min \times 3.12 \times 1000}{13.3 \times 1 \times 0.02 \times 91}$$

$$= \Delta A/min \times 128.9(=130)$$

$$= \text{umoles } min^{-1} g^{-1}$$

where: A/min - Change in absorbance per min of DTNB at a wavelength of 405 nm.

Vol_t = Total assay volume (mL)

Vol_s = Sample homogenate volume (mL) = 0.02 mL

E = Absorbency coefficient (13.3 $cm^2 umole^{-1}$

Lightpath - cuvette width (1 cm)

Tissue conc. = Concentration of brain tissue (mg mL^{-1})

Brain tissue is weighed and 10 times the brain weight in 0.05 M trizma buffer, pH 8, is added to give a ratio of 100 mg per 1.1 mL or 91 mg mL^{-1} (wet weight)

E. Muscle Enzyme Activity (umol/mg/min)

$$\frac{\Delta A/min \times Vol_t \times 1000}{E \times lightpath \times Vol_s \times protein\ conc.}$$

where: ΔA/min - Change in absorbance per min of DTNB at a wavelength of 450 nm.

Vol_t = Total assay volume (mL) = 3.12 mL

Vol_s = Sample homogenate volume (mL) = .02 mL

E = Extinction coefficient (1.33 mM^{-1} or umol mL^{-1})

Cuvette width = 1 cm

Protein conc. = concentration of protein (mg mL^{-1})

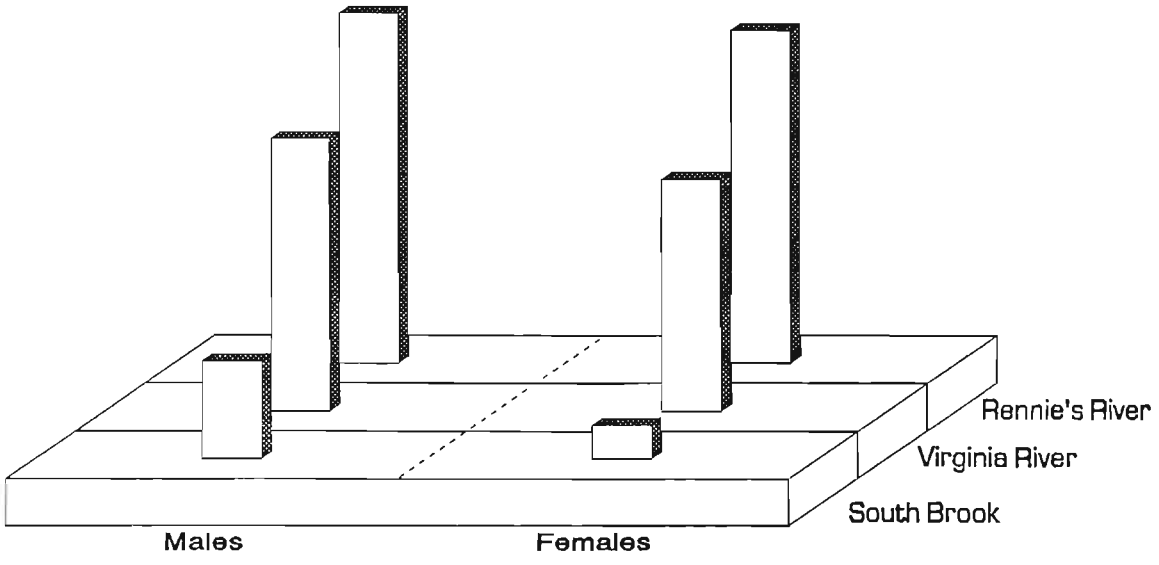
F. Quality Assurance Checks

Brain tissue was pooled and divided into aliquots in individual tubes of homogenate each of which was then run in duplicate on different assay days as a check on assay reliability.

RESULTS

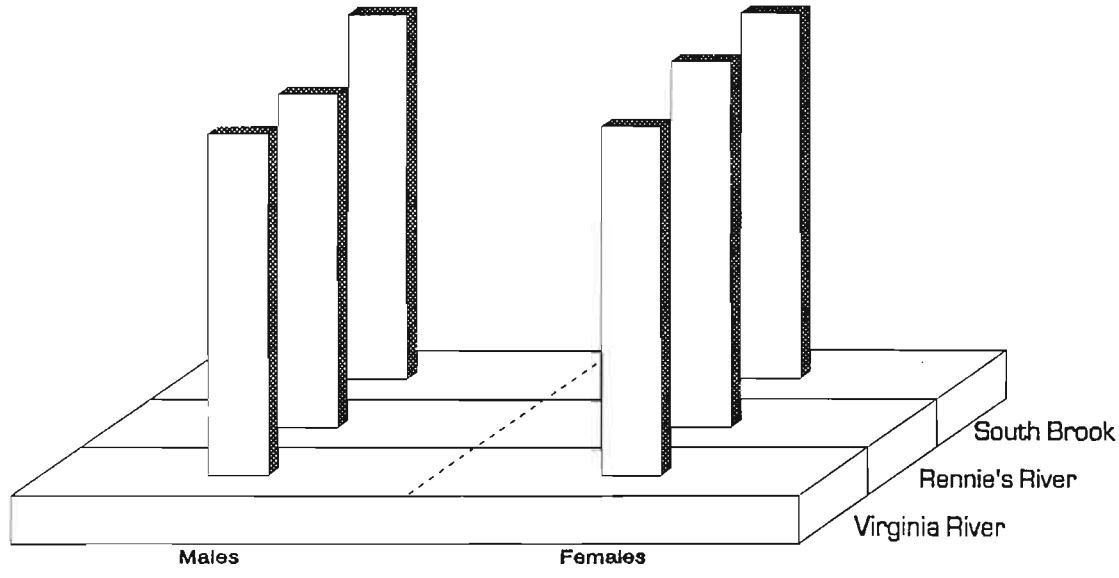
A marked induction of MFO activity was observed in liver tissues of fish from both Virginia River and Rennie's River in comparison with fish from the reference site, South Brook (Figure 1). Male fish from Virginia River were induced approximately 3 fold while fish from Rennie's River were induced 3.6 fold. Higher levels of induction were evident in females, an approximate 7 fold difference being observed in fish from Virginia River and a 10 fold difference in fish from Rennie's River.

With respect to the neurotoxicity indicator, ACE, enzyme levels in brain tissues were similar in both male and female fish from all three rivers (Figure 2). By comparison, evidence was obtained for a distinct inhibition of muscle associated ACE in male and female fish from both Virginia River and Rennie's River (Figure 3). Most striking was the fact that ACE inhibition was observed in the 40-50% range. More detailed information on mean values, standard errors of the mean and F and p values for the various enzyme activities are provided in the Appendix (Tables 1-3).



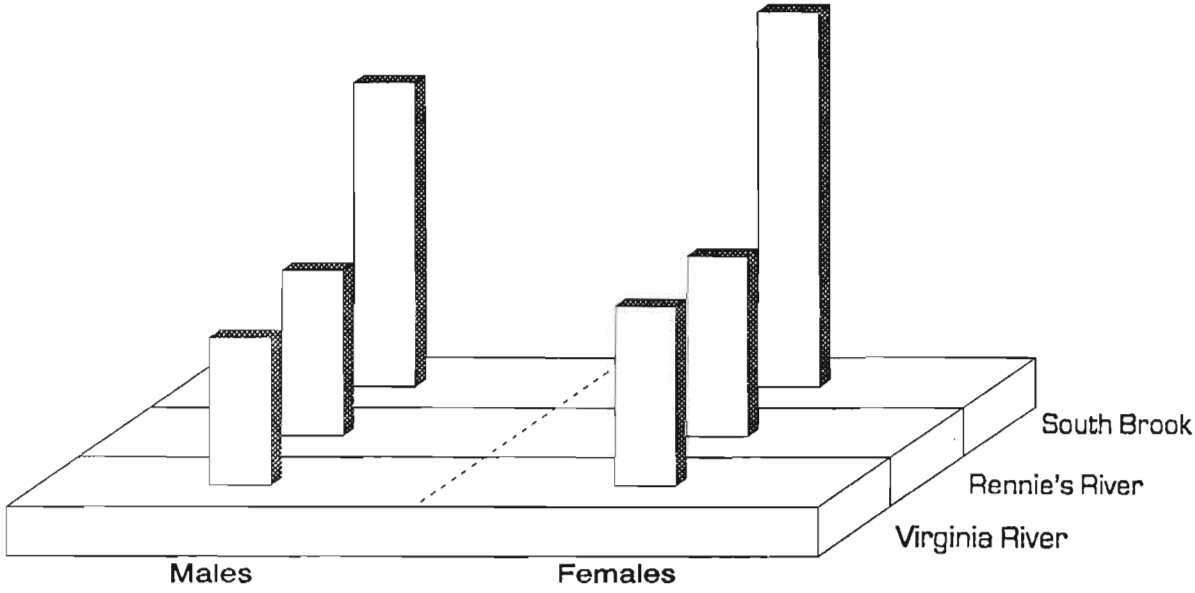
388.3 ± 34.3	368.9 ± 66.6	Rennie's River
302.9 ± 14.9	257.1 ± 66.5	Virginia River
107.3 ± 16.5	37.6 ± 16.1	South Brook

Fig. 1. MFO activity in brown trout liver (pmol/mg/min). Absolute mean values ± SE are given in table. Differences between contaminated and reference sites were highly significant ($p<0.01$).



7.89 ±0.54	7.91 ±0.22	South Brook
7.19 ±0.24	7.91 ±0.35	Rennie's River
7.41 ±0.31	7.56 ±0.39	Virginia River

Fig. 2. ACE activity in brown trout brain (umol/min/g). Absolute mean values ± SE are given in table. Differences between contaminated and reference sites were not significant ($p<0.05$).



0.956 ±0.043	1.184 ± 0.073	South Brook
0.523 ±0.043	0.565 ± 0.056	Rennie's River
0.472 ±0.027	0.569 ±0.037	Virginia River

Fig. 3. ACE activity in brown trout muscle (umol/mg/min). Absolute mean ± SE values are given in table. Differences between contaminated and reference sites were highly significant ($p<0.001$).

DISCUSSION

CAUSE OF MFO ENZYME INDUCTION

MFO enzyme levels from fish in Rennie's River and Virginia River were markedly elevated in comparison with fish from South Brook. The nature of the inducing chemicals in the urban rivers is unknown. As noted above, the chemicals most commonly identified as potent inducers include PCB's, selected other organochlorine pesticides, dioxins and furans and polycyclic aromatic hydrocarbons. St. John's is not industrialized to any extent, and considering the four general types of wastewater, industrial, agricultural, domestic and urban runoff, the latter two are probably the chief sources of contaminants in its rivers. Thus it is reasonable to speculate that from the list given above, combustion derived PAH and used engine oil are likely important inducing chemicals in the rivers. Used engine oil is a common contaminant in urban associated waterways and Upshall et al. (1993) have recently reported on its enzyme induction potential. In this regard, it is worth noting that sediments from St. John's Harbour, which is a major drainage basin for the City and expected to reflect local pollution conditions, contains very high levels of PAH. Organochlorine levels by comparison are relatively low in harbour sediments. (Unpublished report, Department of Fisheries and Oceans Green Plan on Toxic Chemicals.)

SIGNIFICANCE OF MFO ENZYME INDUCTION

The MFO enzyme system plays an important role in detoxification, transforming foreign compounds (which may include many natural dietary constituents) into derivatives more easily eliminated from the organism. However, some compounds can be activated to reactive, more toxic species which may otherwise be generally cytotoxic, mutagenic, or carcinogenic (e.g. see reviews by Bend and James 1978; Stegeman 1981; Conney 1982; Lech et al. 1982; Payne 1984). Most interest to date has centred around a role for MFO in increasing or decreasing certain types of chemical carcinogenesis, but the MFO enzyme system can play a primary role in various types of chemical toxicity, e.g. cataract formation (Nebert and Jensen 1979), retinal degeneration (Hitoshi et al. 1976), atherosclerosis (Majesky et al. 1983), and immune function (Silkworth et al. 1984). There is also recent evidence that metabolic pathways involving cytochrome P-450 enzymes may initiate or modulate damage due to oxygen radicals which are believed to play a key role in a wide spectrum of chemical induced toxicities including carcinogenesis (Gonder et al. 1985). Similarly, because steroid compounds are "natural" substrates for MFO enzymes, physiological functions such as reproduction may be affected in animals subjected to artificially high levels of inducing chemicals (Truscott et al. 1983; reviewed by Khan 1984; Spies et al. 1984). Thus, in view of the mounting evidence for the fundamental importance of MFO in modulating (increasing or decreasing) a variety of toxicological end points, the MFO enzyme activity changes noted in this study are of special interest.

Although elevated levels of MFO enzymes are important for chemical activation, the degree to which chemical byproducts react with important cellular constituents such as DNA depend on levels of other enzymes (so called conjugating enzymes) and biochemicals (such as glutathione), which play a role in deactivation or detoxification (e.g. Payne, 1984). The most important environmental carcinogen and mutagen known to be activated by the MFO enzyme system is the PAH, benzo[a]pyrene. In this regard, it is worth noting that we have been unable to date to provide evidence for the presence of specific benzo[a]pyrene adducts in DNA and protein fractions of trout from the urban rivers (Mathieu and Payne, unpublished observations).

CAUSE OF ACE INHIBITION IN ST. JOHN'S RIVERS

The nature of the ACE inhibiting substances in the urban rivers is unknown. Primary candidates include carbamate and organophosphate pesticides, many of which are potent ACE inhibitors, including in fish (e.g. Lockhart et al., 1985; Morgan et al., 1990). Agriculture is negligible in the water-shed area and if the inhibiting substances are pesticides, the source is likely runoff in association with residential and/or institutional lawn care. Could inorganics be responsible for ACE inhibition? As with most enzymes, relatively high levels of heavy metals are known to inhibit ACE (e.g. Olson and Christensen, 1980). However, the relatively high levels effective in inhibition would be expected to be quite cytotoxic and of little environmental relevance except under very special circumstances (e.g. certain types of "undiluted" mine effluents or similar).

SIGNIFICANCE OF ACE INHIBITION

ACE is necessary for normal functioning of sensory, integration and neuromuscular systems in vertebrates. For instance, inhibition of this enzyme in salmonids has been linked to a variety of functions including respiration (Klaverkamp et al. 1977), swimming (Matton and Lattan 1969; Post and Leisure 1974), feeding (Wildish and Lister 1973; Bull and McInerney 1974), and social interactions (Symons 1973). Mineau draws attention to a quote attributed to Myers and cited by Russell (1981): "Most impressive is the singular fact that acetylcholine is the only substance that can influence every physiological or behavioral response thus far examined".

GENERAL INTERPRETATION

Upon reading the above statements on the significance of MFO enzyme induction and ACE inhibition, one might conclude that the fish in St. John's rivers will, on the basis of MFO enzyme induction, suffer the ravages of cancer, eye cataracts, retinal degeneration and atherosclerosis, or on the basis of ACE inhibition, have troubles with respiration, swimming, feeding and social interaction. All one can say at this time is that the potential for such

maladies is enhanced. This brings us into a "catch-22" situation in interpreting the value of biomarkers. On the one hand, given normal population dynamics, it is known that pollution mediated impacts would likely have to be major before being resolvable in a scientifically credible manner as being due to pollution (witness the problems in accurately estimating the size (and decline) of fish stocks even when it is already known that a considerable proportion of the population is being removed through defined fisheries quotas), on the other hand, individual level responses do not provide a conceptual basis for predicting population level impacts. Both are true. Putting these considerations aside, it might still be argued that individual level responses demonstrate the presence of deleterious substances, and this in itself is important. This can be used as a legal argument (vis a vis the Fisheries Act in Canada) but it is important to note that, from the purview of science, the discipline of toxicology does not recognize the concept of a non-deleterious substance (natural or otherwise)-all substances are toxic with exposure concentrations being all important. Accordingly, any interpretation of individual levels responses can only rely on scientific judgement, which has to take into account the nature of the response, the magnitude of change, the extent to which it occur in a population and animal species under study. For instance, with respect to MFO, finding elevated levels in fish within a few kilometres of an oil-rig site would be very much different from finding elevated levels in fish over a considerable area of the Grand Banks or for instance, in the whole population of beluga whales in the Gulf of St. Lawrence.

For the benefit of the generalist, it is also important to note that basal levels of MFO enzymes in fish and other mammals are not constant. For example, Mathieu et al. (1991) have provided considerable detail on seasonal differences in biotransformation activities in extrahepatic as well as hepatic tissues of striped mullet in the Mediterranean area. An appreciation of such differences in natural variability is important for interpreting any potential toxic effects (direct or indirect) associated with enzyme activities. For instance if there is a 4-5 fold difference in basal enzyme levels in a fish species between season, episodic elevations in enzyme activity caused by contaminants are likely to be of little concern. On the other hand, induction which is both pronounced and prolonged might reasonably be expected to increase the potential for the production of toxicological sequelae.

To return to the present study and conclude. It is reasonable to state that water quality in St. John's rivers is impaired to a sufficient degree to produce early warning symptoms of contaminant stress in fish. Accordingly, any additional loading should be viewed with caution. Projections can not be made about population level effects, but there is a possibility that the health of individual fish is presently being compromised. With respect to water quality improvement, the biomarkers used in this study will be valuable for assessing any remedial measures that may be promulgated by environmental or regulatory interests in the future. In the meantime, it is worth noting that given recent observations on different levels of ACE in the muscle of flounder in association with pollution in the North Sea as well as in brown trout in the present study, the status of this specific enzyme which is a potentially important indicator of neurotoxicity, should be evaluated more fully in the aquatic environment.

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APPENDIX

Detailed information on mean values, standard errors of the mean and F and P values for the various enzyme activities (Tables 1-3).

Male			Female		
Site 2: South Brook	Site 1: Virginia River	Site 3: Rennies River	Site 2: South Brook	Site 1: Virginia River	Site 3: Rennies River
39.0	239.3	239.5	13.2	39.8	316.4
141.7	250.4	280.3	2.0	60.2	325.1
80.2	295.1	262.8	7.7	21.2	382.9
79.3	331.5	286.9	74.3	24.9	30.1
95.7	214.0	352.8	7.6	23.2	556.6
73.8	345.5	92.9	4.3	38.6	529.7
59.5	305.8	450.7	7.3	390.7	441.0
112.6	322.2	394.7	16.8	460.0	
127.8	371.9	383.5	6.6	590.3	
250.6	345.1	443.2	123.8	374.5	
68.9	311.6	430.0	149.8	520.2	
158.4		369.0		177.0	
		674.0		622.1	
		538.5			
		436.5			
		620.2			
		345.4			
n 12	11	17	n 11	13	7
$\bar{x}$ 107.29	302.94	388.29	$\bar{x}$ 37.58	257.136	368.96
SE 16.51	14.89	34.28	SE 16.08	66.549	66.56
F-value	76.43	42.18	F-value	8.79	35.04
p-value	0.000	0.000	p-value	0.007	0.000

Table 1. Liver mixed function oxygenase activity (pmol.mg^{-1}.min^{-1}) in brown trout

Male			Female		
Site 2: South Brook	Site 1: Virginia River	Site 3: Rennies River	Site 2: South Brook	Site 1: Virginia River	Site 3: Rennies River
5.98	8.19	5.59	7.67	8.09	6.92
7.90	7.02	8.71	9.17	6.37	7.51
6.83	5.36	7.12	7.12	7.31	9.36
13.46	7.80	7.61	8.39	7.70	6.83
7.70	7.51	8.09	8.97	7.90	8.19
7.22	7.31	6.83	7.70	4.58	7.80
7.80	7.31	8.78	7.12	9.65	8.78
5.75	6.73	6.44	7.51	8.65	
7.41	8.58	5.85	8.29	8.00	
9.00	6.53	7.12	7.80	9.46	
8.19	9.10	5.56	7.22	6.73	
6.44		7.51		8.00	
8.87		8.00		5.85	
		7.70			
		7.51			
		7.80			
		6.24			
n 13	11	17	n 11	13	7
$\bar{x}$ 7.89	7.40	7.20	$\bar{x}$ 7.91	7.56	7.91
SE 0.54	0.31	0.24	SE 0.22	0.39	0.35
F-value	0.55	1.58	F-value	0.54	0.00
p-value	0.466	0.220	p-value	0.472	0.985

Table 2. Brain acetylcholinesterase activities (umoles.min^{-1}.g^{-1} (wet weight)) in brown trout

Male			Female		
Site 2: South Brook	Site 1: Virginia River	Site 3: Rennies River	Site 2: South Brook	Site 1: Virginia River	Site 3: Rennies River
.592	.561	.338	.998	.761	.414
1.098	.338	.494	1.301	.458	.473
.902	.375	.528	1.240	.553	.477
.851	.464	.237	.818	.638	.452
1.017	.420	.437	1.518	.469	.785
.932	.591	.378	1.561	.352	.724
1.028	.615	.549	1.041	.473	.633
1.033	.433	.362	1.020	.575	
.936	.430	.421	1.412	.615	
1.146	.442	.531	1.111	.679	
.942	.525	.698	1.000	.608	
1.156		.443		.800	
.797		.870		.433	
		.634			
		.774			
		.797			
		.391			
n 13	11	17	n 11	13	7
$\bar{x}$ 0.956	0.472	0.523	$\bar{x}$ 1.184	0.570	0.565
SE 0.043	0.027	0.043	SE 0.073	0.037	0.056
F-value	84.43	49.01	F-value	62.76	36.96
p-value	0.000	0.000	p-value	0.000	0.000

Table 3. Muscle acetylcholinesterase activities (umol.mg^{-1}.min^{-1}) in brown trout