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Biochemical effects of a pollutant gradient – introduction

R. F. Addison

Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, Canada B2Y 4A2

Many biochemical techniques have been developed during the last few years to allow the detection of the effects of various pollutants on aquatic biota. Some of these are based on clinical chemical approaches originally developed to assess human health; others are derived from basic studies of the mechanism of action of specific toxicants (usually in mammalian systems). However, of all the approaches available, only a very few have shown any promise as techniques with which to assess the effects of pollution in the field. These are: (1) induction of enzymatic detoxification systems by certain organic contaminants; (2) induction of metallothionein by certain heavy metals. Undoubtedly, some of the success of these 2 approaches arises from the fact that they are, first of all, specific to a relatively small suite of pollutants; they are therefore likely to be insensitive to stress caused by factors other than pollution, such as temperature or salinity changes, disease, etc. (though the indices measured may vary with natural biological factors). Secondly, both approaches depend on detecting an *increase* rather than a depression of the variable being measured, and this simplifies the analytical chemistry involved. Finally, both approaches are based on a large body of information from mammalian studies, which provides a basic understanding of the mechanisms underlying the cause-effect relations.

Studies of drug metabolism in mammals carried out during the 1950's and 60's showed that an enzyme system existed in liver which was associated with the endoplasmic reticulum, required oxygen, involved a specific cytochrome (P-450, so-called from the absorption spectrum of its CO complex) and was inducible on exposure of the organism to various organic compounds. This enzyme system, 'mixed function oxidase' (MFO) or, more recently, 'mono-oxygenase', generally catalysed the conversion of lipophilic substrates to more polar products ('Phase I' reactions); these intermediates could be conjugated (by 'Phase II' enzymes) to excretable products. In the 1970's and 80's, fish were shown to contain such enzyme systems, which were inducible by certain organic pollutants, including some chlorobiphenyls and polynuclear aromatic hydrocarbons. Activity of the 'Phase I' enzymes in fish is now usually measured with substrates such as ethoxyresorufin or benzo(a)pyrene, which are converted to products which can easily be analysed with a high degree of specificity and sensitivity.

Hepatic mono-oxygenase induction in teleost fish has been used successfully on many occasions as an 'effects monitoring' technique, and the papers which follow provide evidence of its success at the GEEP Workshop also. Mono-oxygenase induction in invertebrates is less well established as a monitoring tool though its potential was also examined in some detail at the workshop. In addition, the possible application of some 'Phase II' enzymatic measurements as indices of pollutant exposure was evaluated.

Early studies on the toxicity of metals to fish showed that fish could acclimatise to increasing concentrations of some heavy metals. This led to the recognition that there existed in both vertebrates and invertebrates a group of proteins whose function was mainly to bind metals, usually those of Group IIB. The general characteristics of metallothionein are that it is of low molecular weight (ca 6,000–12,000 depending on species and tissue), it contains relatively high concentrations of cysteine residues (up to 30 residue %), and it is inducible on exposure of the organism to certain metals. Metallothionein is usually determined via its bound metal content (measured, for example, by atomic absorption) after a preliminary fractionation of tissue homogenate on a molecular weight basis (e.g. by gel filtration). This section ends with descriptions of metallothionein measurements in fish and invertebrates, studied at the workshop as potential 'effects monitoring' tools.

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