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# EFFECTS OF POLLUTANTS ON AQUATIC ORGANISMS

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# PREFACE

Most fields of scientific endeavour change in direction and technical approach during the course of time. Such a shift in emphasis is currently apparent in pollution studies, the earlier overwhelming concentration on lethal levels and degree of accumulation of toxic materials by organisms now being matched by experimental investigations into the more subtle influences of potential pollutants.

On 11 April 1975 a seminar was arranged at Liverpool University under the auspices of the Society for Experimental Biology, at which a number of the leading exponents of the experimental approach to pollution forgathered to outline the current state of the art. Their lectures, including material on such wide-ranging aspects as the pathways and rates of uptake, detoxification and release from the body of foreign substances, effects of toxins on specific physiological systems and adaptation of organisms to chronic low levels of pollutants, are now assembled in this volume. Coverage is such as to include mention of most of the substances currently considered to have significant effects on aquatic organisms, and the authors have in general included some background review material to put their contribution in perspective. Whilst it is impracticable to give a comprehensive account in a book of this size, the variety and breadth of approach will hopefully provide an introduction to this rapidly expanding field suitable for undergraduates and research students. The numerous and obvious gaps in knowledge made apparent may in addition serve to stimulate other scientists to interest themselves in this area.

Advice from many sources was received during the planning and execution of the seminar and, in addition to the speakers, I would like to thank Professor R. Fänge, Dr G. Howells, Dr J. S. Alabaster and Dr I. C. White for suggestions on contributors; the SEB Zoological Secretary Dr P. Spencer Davies for administrative assistance and Dr T. A. Mansfield for his efforts in successfully garnering the funds necessary to mount the seminar.

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*July, 1975*

A. P. M. Lockwood
*Editor for the Society for Experimental Biology*

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GWYNETH HOWELLS

# Introduction

Introducing this volume, which focuses on experimental studies in the aquatic environment, provides me with an opportunity to raise some issues that may perhaps stimulate discussion. Increasing concern for environment and pollution has been influential in changing attitudes to pollution in UK as in other countries, and this is demonstrated by provision in legislation recently enacted, which will strengthen official demands for monitoring and surveillance schemes designed to control unwelcome emissions.[1] Further, as a partner in the European Economic Community we are being pressed to accept quality standards – at least initially for drinking water and foods and perhaps, at a later stage, environmental quality standards. It is also true to say that the improving conditions (e.g. of air and river quality in London) induce further popular expectations of improving quality which are hard to deny, even when economic cost is high.

It is worth reiterating at this stage the sequential steps involved in recognising pollution, setting up relevant investigations and instituting control or management procedures. These are set out in Fig. 1.

In the first place, recognition of 'pollution' surely depends upon the observation of environmental or biological damage and this leads us to identify the toxic agent or contributing circumstances. We can then begin to make measurements of the damaging and background concentrations to which organisms are exposed. At this stage surveillance or monitoring programmes and control measures are sometimes set up, although without an understanding of dose/response relationships, of mechanisms of toxicity, or assessment of the overall risks, these can only be accepted if justified by the gravity of the circumstances.

I now wish to examine in more detail some of the other contributions to pollution studies that are set out in Fig. 1.

Many problems come to mind: How can monitoring schemes be designed? Should they simply be instruments of control (is the emission acceptable; below a set concentration and volume, or not)? How will toxic concentrations be related to effects? How will ecological effects be measured? How will

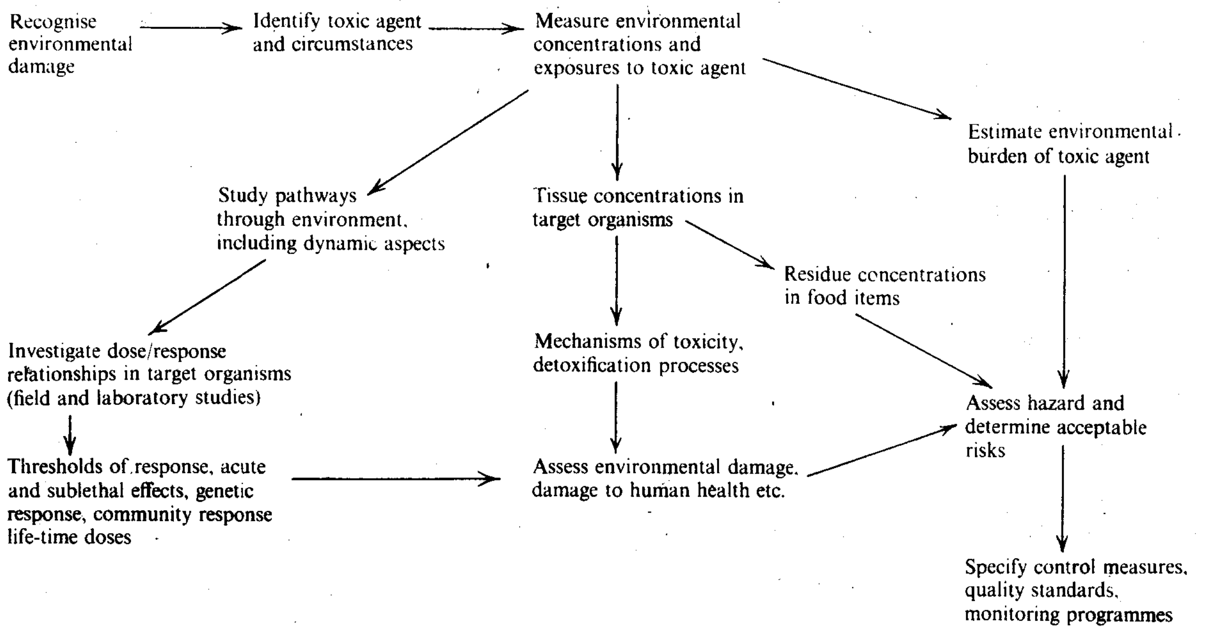


**Fig. 1. Scheme of pollution investigation and assessment.**

especially sensitive species or populations be protected? What are the criteria of 'acceptable' risk or damage? These questions are not simply a matter for scientific endeavour, common-sense or administrative judgement – they call for an amalgam of all these, and probably many other, approaches.

Once we have observed an effect and related it to a particular toxic agent, it is customary and practical to use sensitive physical or chemical analysis to determine the levels of concentration. However, danger lies in the increasing sophistication and sensitivity of these techniques which engender the view that accurate measurements and an impressive array of decimal places are to be associated with significance. A paramount need is to relate these measurements (concentrations) to their effects in a quantifiable and predictable way, including measurement of length and mode of exposure (dose), so that an assessment can be made of whether a particular observed level is one to elicit remedial action, preventive measures or other control sanctions. A difficulty is that while some pollutants have no natural function (e.g. the man-made element plutonium) many others are present naturally, or even have an essential role and are toxic only because they are present in the 'wrong' form, place or concentration, so that simple detection of their elemental presence may be irrelevant.

Dose/response relationships call, in the first instance, for detailed laboratory studies involving different life stages of organisms and different and varying external conditions and analysis of synergistic or antagonistic reactions. We know a little about the dose/response relationships of only relatively few toxic agents and target organisms and, with present and foreseeable scientific effort, only limited advances can be made towards the ideal state of knowledge.

Moreover, pollutants are seldom presented in real life in the way in which we usually design experiments, i.e. with exposure at a constant concentration. The common pattern of pollutant exposure in the field is to fluctuating rather than constant concentrations, allowing opportunity for recovery, adaptation or simple avoidance of stress, provided the exposure concentration is not acutely toxic. In the case of low subacute exposure, assessment of the integrated effect over the life span is needed, not just an averaged exposure applied for the whole life span.

Responses of individuals (usually the concern of the experimenter and the toxicologist) need to be distinguished from community responses (the concern of the ecologist and the epidemiologist). In the first case, 'averaged' responses are usually irrelevant in the assessment of individual risk, although a 'norm' against which to judge response is a valuable reference standard. Further, although some biological phenomena observed in a group or population can be represented by a normal or log-normal distribution of points, as many examples of non-Gaussian distribution could be cited. Lack of an observed response could thus be because it is below the levels of detection or recognition, because there is a threshold of response, or because the sensitivity of the target organism is anomalous.

Indeed the uncharacteristic sensitivity of a few individuals has the most important implications for species survival and examples of 'naturally' selected tolerant varieties (e.g. sulphur dioxide tolerant Helmshore ryegrass, copper tolerant *Ectocarpus*) are recognised, and in some instances can be genetically distinguished.

Community response assessed in terms of averaged exposure is easier to accept, in that it is the overall status or health of the community that is important. Nevertheless, the survival of a few, more tolerant individuals in a community has provided the means of population recovery when accidents have seemed, at first sight, to have brought about total destruction.

Clearly there are so many complex issues that some rationalisation and selection has to be made if scientific effort is to be focused on major problems. It has been suggested [2] that of eighteen identified 'environmental pollutants' only eight could be considered as potentially hazardous (to man); a critical reappraisal of candidate toxic agents could help to establish a priority listing so that scientific effort could be directed to the most pressing problems. Too often the demand for control of a pollutant appears to be determined by political feasibility, expedience, emotions of the moment, and on unsupported speculation rather than on hard scientific facts. This may cause some suspected agents to be controlled unnecessarily rigorously, while others may be equally unjustifiably exonerated. There is no real substitute for acquiring the relevant information, at whatever level of detail seems appropriate, from both experimental and field observations. Furthermore, if the estimated degree of damage is required to implement control (e.g. if it is to be used in a legal sense to assess 'damages'[3]), or to estimate potential for recovery, some quantitative measure of effect has to be made.

The prospect of studying a wide variety of materials, at a range of concentrations, in conjunction with all environmental variables as well as other pollutants, on all life stages of all possible target organisms, in both acute and subacute dosages, is clearly impracticable. No nation could justify putting so much scientific effort to such an endless task, and ways to limit endeavour to crucial aspects have to be sought. It is salutary to consider the disciplined approach made by toxicologists with man as the prime target, and by radiation health physicists to analyse critical pathways.

Fortunately, we have recourse to some kinds of investigation which although presenting problems of their own, and equivocal if left to stand alone, can help to evaluate hazards and to relate laboratory studies to environmental conditions. First, field studies can be made of existing discharges and the biological observations related to measured gradients of exposure concentration. These investigations have the advantage that contemporaneous conditions and interactions are taken into account, but difficulties of interpretation arise if exposure fluctuates, even when the fluctuations can be recorded. Secondly, investigation of accidents or incidents when excessive quantities of a toxic agent have been released can be revealing not only about the effects of a sudden acute and high-level exposure (and its long-term consequences), but also about the course and time of recovery. Case histories of these occasions are a valuable source of information. Thirdly, continuing surveillance of biological 'status' coupled with water quality or similar type information, can be used to document long-term trends, even where levels of exposure are too low for direct observation of acute effects. These studies, in particular, take account of interspecies interactions, life history and lifetime effects, and can be used to signal unsuspected hazards. Real problems are recognised, however, in selecting valid methods of sampling where discontinuous or sparse populations occur, in determining the level of species identification needed (genus, species, variety) and in data handling (diversity indices)[4]. A parallel may be drawn with epidemiological studies, which although difficult to set up and maintain, can nevertheless provide valid conclusions and risk assessments.

The most promising path lies surely in developing a fruitful interaction between laboratory and field investigations – field observations such as those described, leading us to ask questions that can be answered, albeit only partially, in the laboratory. We look to experimentation to simulate or enhance environmental conditions while excluding or controlling other contributory factors, or to manipulate field conditions, as well as to record and quantify responses. In turn, results of experimental studies should lead to hypothesis and prediction about the environment which can only be confirmed or rejected by further field study.

Most scientists, in the course of their careers, develop interests directed towards either the field or the experimental approach and we should be cautious of conclusions about the living world derived from a single aspect of scientific endeavour. I hope that the initiative shown by the Society for Experimental Biology in promoting this Seminar will help to generate the reaction between these two kinds of investigation.

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G.W.BRYAN

# Some aspects of heavy metal tolerance in aquatic organisms

In most waters the concentrations of heavy metals are very low (Riley & Chester, 1971), although higher natural concentrations occur in rivers and estuaries which are associated with outcropping metalliferous lodes. As a result, the concentrations of heavy metals in natural waters can easily be increased to levels which aquatic organisms have not previously encountered. The mechanisms possessed by organisms for handling natural fluctuations in the availability of heavy metals assume particular importance under contaminated conditions. The degree of protection afforded varies from species to species, so that under contaminated conditions the ecological balance may be tilted as the more tolerant organisms are favoured. From the public health point of view, these protective mechanisms determine the degree of contamination of edible fish and shellfish, even if the organisms themselves are unaffected.

This paper is not primarily concerned with the manifold toxic effects of heavy metals but deals with some of the processes which determine the tolerance of aquatic organisms to heavy metals.

## Absorption of heavy metals

### *Absorption from solution*

Movements of heavy metals which are attributable to active transport systems have largely been observed in unicellular organisms. In yeast, for example, arsenate is absorbed by and can ultimately inhibit the phosphate transport system (Chan & Rothstein, 1965) and zinc, cobalt and nickel are absorbed by a system which also transports magnesium and manganese (Fuhrmann & Rothstein, 1968). Absorption of nickel also appears to be an active process in the ciliate *Paramecium caudatum* (Andrivon, 1970, 1974) and in the embryo of the sea urchin *Lytechinus pictus* (Timourian & Watchmaker, 1972). It is not difficult to imagine that in higher organisms movements of heavy metals might be mediated by carrier systems used primarily for calcium or magnesium, but absorption of zinc by the liver of the puffer fish *Tetraodon hispidus* was shown to be a passive process (Saltman & Boroughs, 1960).

Pinocytosis by animal cells is quite common and has been implicated in the absorption of colloidal gold by the mantle cells of bivalve molluscs (Bevelander & Nakahara, 1966) and in the uptake of vanadium bound to mucopolysaccharides by the pharyngeal cells of ascidians (Kalk, 1963).

For the most part, the uptake of heavy metals by aquatic plants seems to be a passive process, although one which can be influenced indirectly by metabolism. Davies (1973) showed that the kinetics of zinc uptake by the diatom *Phaeodactylum tricornutum* could be explained by the rapid adsorption of zinc onto the cell membrane, followed by diffusion controlling the rate of uptake and binding to proteins within the cell. Binding to protein may control the concentration in the cell, because, during the growth cycle, the concentration of zinc reaches a maximum and then decreases as the amount of protein in the cell declines. A similar pattern has been observed for nickel in the same species (Skaar, Rystad & Jensen, 1974). It was also shown that in phosphate-starved cells the capacity for nickel absorption was low and was enhanced by pre-treatment with phosphate, presumably due to the synthesis of new binding sites.

In seaweeds, adsorption or ion-exchange processes are involved in the uptake of zinc, since the amount absorbed at different external concentrations was described by the Freundlich adsorption isotherm in the green alga *Ulva lactuca* and in the red alga *Porphyra umbilicalis* (Gutknecht, 1961, 1963, 1965). It was also shown that uptake was promoted by high pH, temperature and, in most species, light, although in the brown weed *Fucus vesiculosus* light decreased absorption. It is thought that relationships between uptake and light can be explained in terms of an indirect effect of photosynthesis on the internal pH of the plant and on the synthesis of more binding sites.

Absorption from solution by most animals seems to involve passive diffusion of the metal, probably as a soluble complex, down gradients created by adsorption at the surface (cuticle, mucus layer etc.) and binding by constituents of the surface cells, body fluids and internal organs. This pattern appears to hold for zinc in fish eggs (Wedemeyer, 1968), fish (Pentreath, 1973*a*), euphausiids (Fowler, Small & Dean, 1969), decapod crustaceans (Bryan, 1971) and polychaetes (Bryan & Hummerstone, 1973*a*). Relationships between rate of absorption and external concentration for several metals in the polychaete *Nereis diversicolor* are shown in Fig. 1. The rate of absorption of manganese is directly proportional to the external concentration, but for other metals the relationship is less exact. In the case of zinc, this is because uptake is more closely related to its adsorption on to the surface of the body during the uptake process. Direct proportionality between uptake rate and

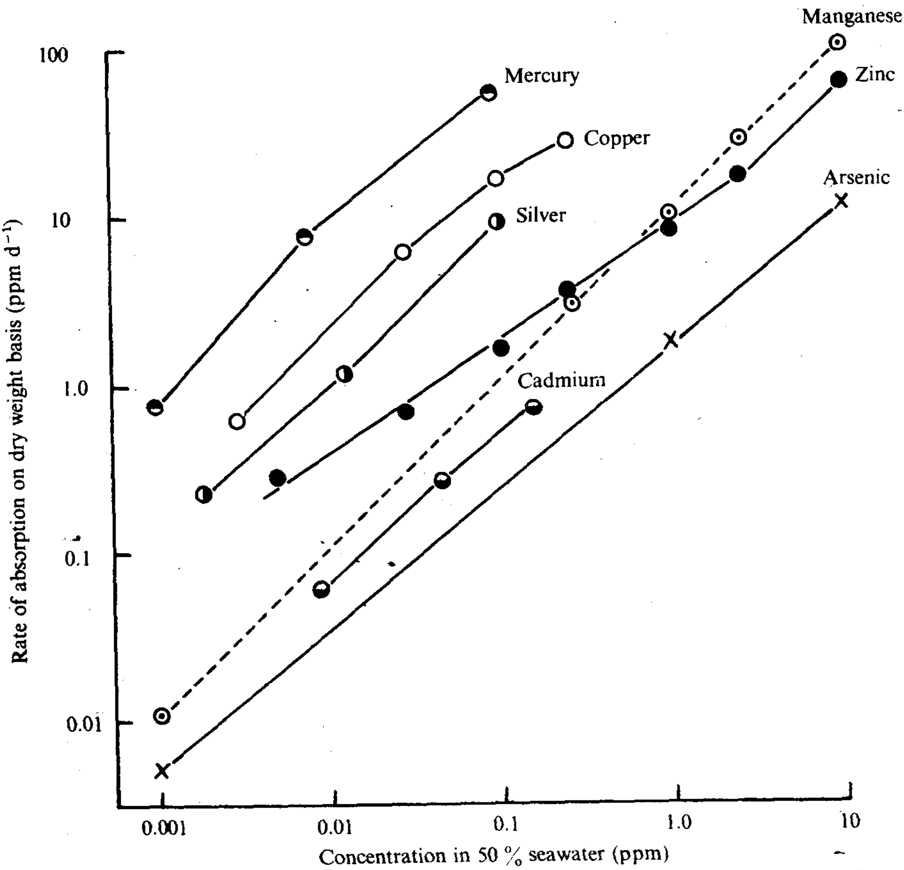


Fig. 1. Rates of absorption of metals from different concentrations in 50 % seawater by *Nereis diversicolor* from Avon estuary (Devon). Each point is a mean value for five worms and measurements were made using radioisotopes at 13 °C. Mercury was added as chloride, silver as nitrate, arsenic as arsenate and others as sulphates.

concentration has also been observed for manganese in the polychaete *Hermione hystrix* (Chipman, Schommers & Boyer, 1968), for cadmium in the shrimp *Lysmata seticaudata* and the mussel *Mytilus edulis* (Fowler & Benayoun, 1974), for chromate in the bivalve *Tapes decussatus* (Chipman, 1966) and for chromate and lead in the oyster *Crassostrea virginica* (Shuster & Pringle, 1969). There is no evidence that any animals can prevent the entry of metals by changing the permeability rapidly, although organisms such as bivalve molluscs can temporarily prevent absorption by closing the shell.

The permeability of various species is of considerable importance in determining their tolerance to metals and in *Nereis* (Fig. 1) there is a fairly close