CHEMICAL DISRUPTION OF BIOLOGICAL PHENOMENA

Victoria V Cheung, Tamara S Galloway
Plymouth Environmental Research Center, University of Plymouth, Drake Circus, Plymouth. UK.

Michael H Depledge
Environment Agency, Rio House, Aztec West, Bristol, UK.

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Contents

Summary

The input of chemicals produced by human activity into the environment, many of which eventually reach the aquatic environment, is of great concern. In recent times, certain groups of chemical contaminants have been linked to specific biological effects and have been found to be able to disrupt biological functions. With the formulation of ever increasing numbers of new chemicals, there is a continuing need to monitor the pathways and effects of such xenobiotics. These have included pesticides which have inadvertently caused disruption in endocrine systems, agents which damage the DNA and are genotoxic, and contaminants which have detrimental effects upon immune systems. Ecotoxicology is a science that integrates the ecological and toxicological effects of chemical pollutants on populations, communities, and ecosystems with the fate of such pollutants in the environment.

Here a brief overview of the biological effects of chemical exposure is presented. Examples of sublethal biological effects and the different classes of chemicals present in the environment that may cause them are described. The application of biomarkers in environmental monitoring programmes is discussed and their role and usefulness for meeting regulatory guidelines are outlined.

1. Introduction

There is no doubt that with the proliferation in human populations, anthropogenic actions such as increases in shipping activities, the growth of aquaculture production, the expansion of tourism activities and resource exploitation has resulted in a continual increase in the production, consumption and disposal of chemicals into the environment. It has been estimated that approximately 70% of the human population resides within 60 km of the coastal zone and a significant proportion of the world's largest cities are connected either directly to an estuary or located further up river from an estuary. Different pollutants of varied origin, from terrestrial to air pollutants, often eventually reach the marine environment. With the world's coastal zones being its most productive habitats, considerable effort is required to monitor the pathways, bioavailability and fate of contaminants in the aquatic environment. Such pollutants are able to interact with ecologically sensitive systems and the effects may be observed at various different levels of biological organization.

The intrinsic physico-chemical properties of a toxic substance affect its fate and behavior within organisms and ecosystems. Properties such as the molecular structure, aqueous solubility and vapor pressure determine the rates and pathways by which chemicals move among environmental and biological compartments. The chemical structure of a contaminant determines its stability and persistence in the environment; it also influences the mechanism and degree of toxicity. The lipid solubility of an organic chemical often correlates with the degree of bioaccumulation. It is therefore necessary to develop, validate and apply rapid methods to measure the biological impacts of chemical contaminants in the environment and assess the risk associated with the exposure to such xenobiotics.

The effects of a chemical contaminant on an organism can be observed at the various biological levels of organization. The lowest levels of organization being the biochemical and subcellular, followed by the tissues and organs, whole organisms, and at the highest levels, those being the population and ecosystem levels. In principle, ecotoxicological tests can be carried out at any biological level. However, tests become easier to control and reaction times decrease, thus reproducibility, reliability, robustness and repeatability all increase, moving down from the ecosystem to the molecular level. Figure 1 represents how chemical contaminants can have effects at different biological levels over time. The basis of all xenobiotic-induced injury is the initial perturbation of biochemical and molecular processes within the cell, which may proceed onto higher levels of organization with time and level of exposure. Ultimate effects can include extensive changes in distribution or age structure or species and alterations to the function of the ecosystem.

In the early phases of the environmental monitoring of coastal areas, most programmes have consisted of the measurement of physical and chemical variables, and only occasionally have biological variables been taken into consideration. Such programmes have given useful information regarding levels of contamination but with the exception of the qualification and quantification of benthic fauna found from sediment grabs, have provided relatively little information on the effects of contaminants on the biota. In the 1960s, there was concern over the effects of organochlorine chemicals in the marine environment, in particular DDT and PCBs. The routine measurement of such contaminants in the water column however, could not always be carried out since the

concentrations were often below the level of detection of chemical instruments. With the realization that herbicides, pesticides and antifouling agents are likely to cause effects on the marine environment, more attention has turned towards the monitoring of biological effects rather than contaminants. Researchers have since concentrated on the development of methods that could provide an early warning of effects on biota caused by the wide variety of contaminants present in the marine environment. Such indicators have been termed 'biomarkers'.

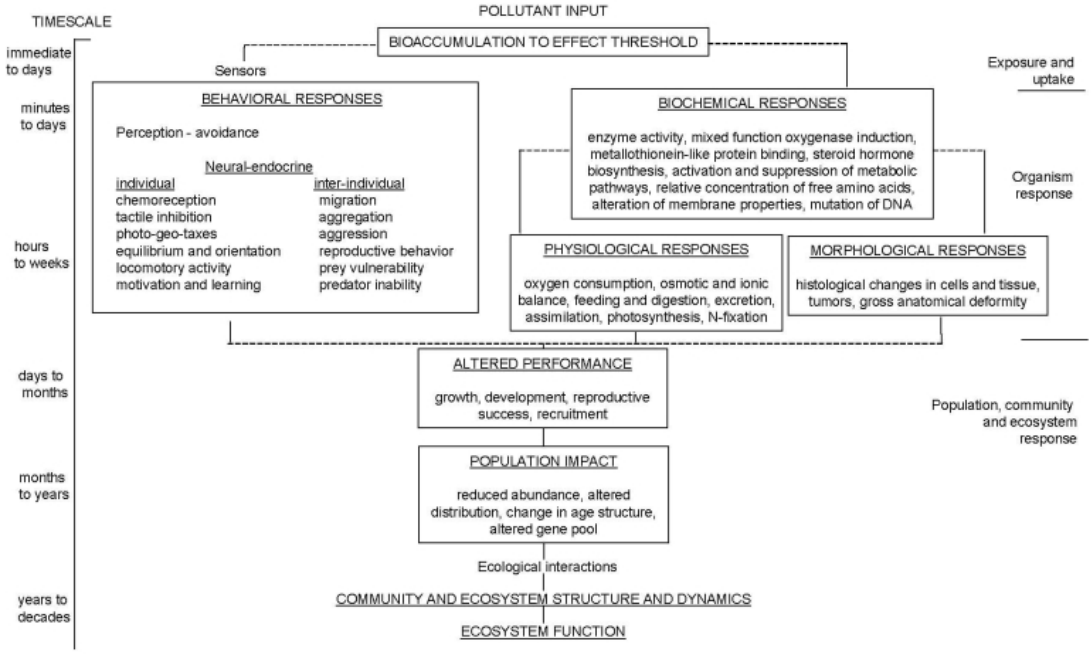

Figure 1. A chronological diagram representing the biological impacts of toxic pollutants, emphasizing responses at different levels of biological organization.

Biomarkers are measurable biological parameters that change in response to xenobiotic exposure and other environmental or physiological stressors, and can be indices of toxicant exposure or effects. There are significant advantages to applying biomarkers to complement traditional chemical methods of detecting contaminants:

- Biomarker responses may indicate the presence of a biologically available contaminant, or form of a contaminant, rather than a biologically inert form of the chemical.
- The application of a suite of biomarkers may provide the evidence for the presence of a contaminant not initially suspected.
- Biomarker responses may be observed long after a transient exposure to a contaminant which has since been degraded or modified and might therefore no longer be detectable. Therefore, biomarkers may provide indications of intermittent pollution events that may be missed by routine chemical monitoring measured at specific times.
- The application of biomarkers are often much simpler to perform and considerably less expensive than a wide range of chemical instruments and methods of analyses.

It has been suggested that some biomarkers are potential surrogate measures of chemical contaminants. For example, in place of the measurement of trace metal concentrations in environmental samples, the concentration of the metal binding protein metallothionein may be measured in the tissues of organisms that have been exposed to trace metals. Ecotoxicological research into the use and application of biomarkers provides the basis for strategies of risk assessment and for improved environmental decision-making.

2. Biological Responses to Chemicals in the Environment

There are a number of different types of wastes that result from human activities, many of which are not subject to bacterial attack (unlike organic material that tends to be degradable) and therefore are effectively permanent additions to the environment. Such persistent contaminants are termed 'conservative wastes'. The three principle categories of such wastes are:

- Heavy metals (e.g. mercury, copper, lead, zinc etc.).
- Organics (e.g. chlorinated hydrocarbons, polychlorinated biphenyls, polycyclic aromatic hydrocarbons etc.).
- Radioactivity (i.e. ionizing radiation).

Each of these classes of contaminants may interact with biological material through a variety of mechanisms. The responses to chemical contaminants may be observed at the various levels of biological organization (as illustrated in figure 1). Changes resulting from cell injury may be divided into two phases: a 'reversible phase', which is associated with sublethal cell injury, and may proceed onto the second 'irreversible phase'. The irreversible phase is reached when the injurious stimulus is removed, and the cell is unable to recover even though it has been returned to a normal environment. These sub-cellular interactions may lead to deterioration of the health status of organisms; detrimental effects on the reproductive output or success; or in cases where the biological effects are non-repairable, death of the organism. Such changes in cell function and pathology caused by pollutant exposure can be used as early warning signals of possible injury at higher levels of biological organization.

2.1. Biological Effects of Heavy Metals

It is difficult to quantify the input of metals into the environment caused by anthropogenic activity since natural sources of metals occur from the erosion of ore-bearing rocks, wind-blown dust, volcanic activity and forest fires. Inputs of metals due to human activity are by atmospheric deposition, via rivers and from direct discharges or dumping. Agricultural sources of metals are equally as extensive as industrial sources, with metals occurring in significant quantities in metal-stabilized or derivative pesticides and insecticides, sewage and agricultural wastes, sludges and slurries, and inorganic fertilizers. Furthermore, the dredging of contaminated harbors and estuaries is a significant source of metals in some areas and result in the remobilization of metal contaminants. The interactions of heavy metals in the environment are summarized in figure 2.

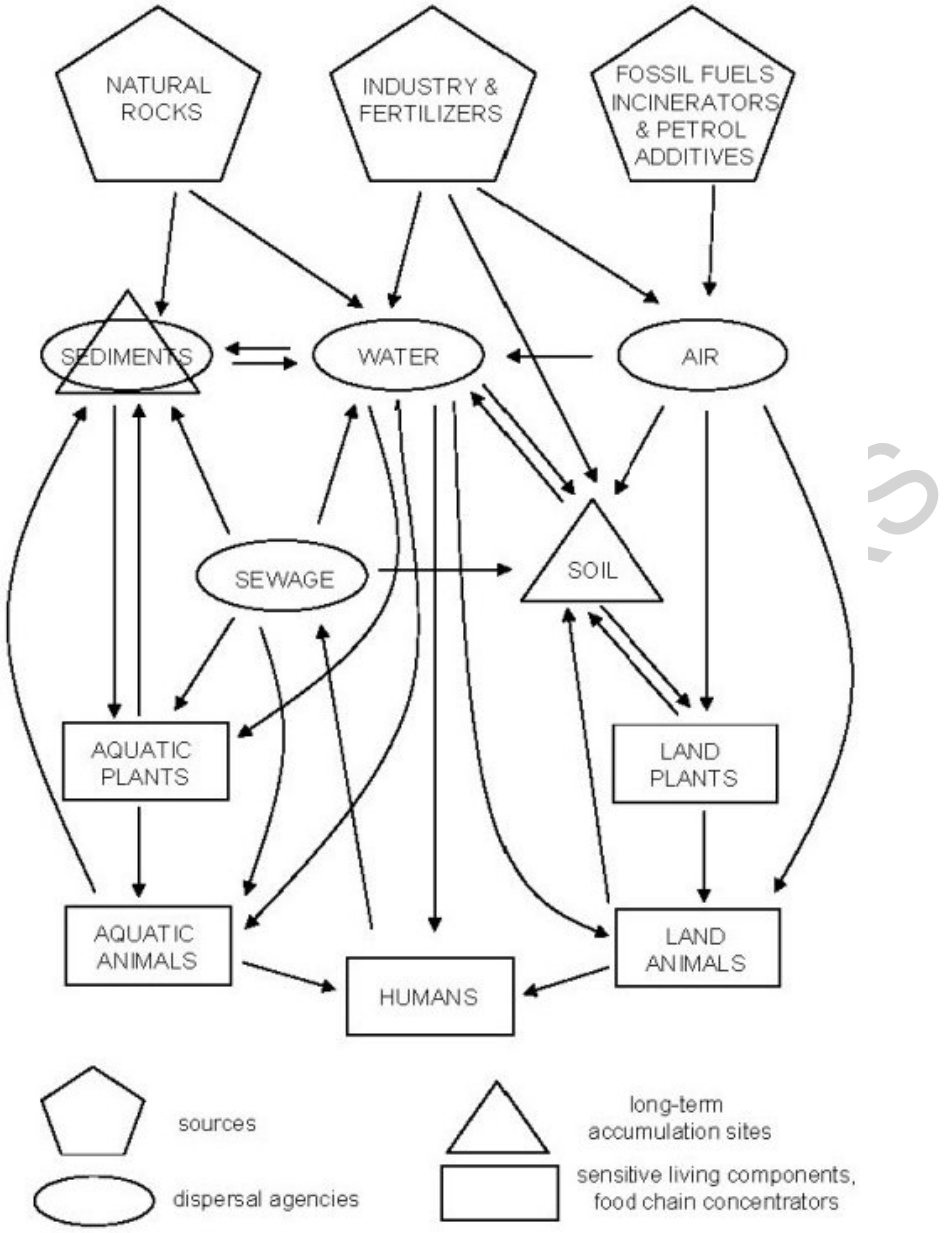

Figure 2. Heavy metal interactions in the environment

The presence of a metal species in the environment does not necessarily constitute a threat to the health of an organism. Indeed, the presence of certain amounts of some metals is indispensable to the living cell. For example, the essential metals iron and copper are required in various respiratory pigments and in oxidative enzyme systems required in metabolism, and the major inorganic elements sodium, potassium, calcium, magnesium, silicon and chlorine have important biological roles. However, in excess such essential metals can be toxic, furthermore there are a number of non-essential metals that are also considered to be toxicants. The ability for plants and animals to regulate their metal content is usually limited and metals that cannot be excreted are often bioaccumulated. Organisms of higher trophic levels feeding upon bioaccumulators

will have a diet enriched by these contaminants and may accumulate a greater body burden hence bioaccumulation occurs going up the food chain.

Metals have been associated with inducing oxidative DNA damage, teratogenicity and cancer. One of the reasons for this carcinogenic potential is the catalytic activity of redox-active metals, including iron and copper which may result in the production of hydroxyl radicals. In addition, the inherent DNA-binding properties of such metals bring freshly generated and highly reactive hydroxyl radicals in close proximity to the molecular target, the DNA (figure 3). Many heavy metals have been shown to act specifically by inhibiting certain enzymes, thus interfering with metabolic processes in development. Divalent cations are able to cross-link sulfhydryl groups and in so doing disrupt the tertiary structure of enzymes rendering them less active or inactive. Such alterations in activity may result in teratogenic responses, or less dramatic effects may be observed such as retarded rates of growth, inhibited, delayed or precocious hatching of embryos, or mortality of the organism.

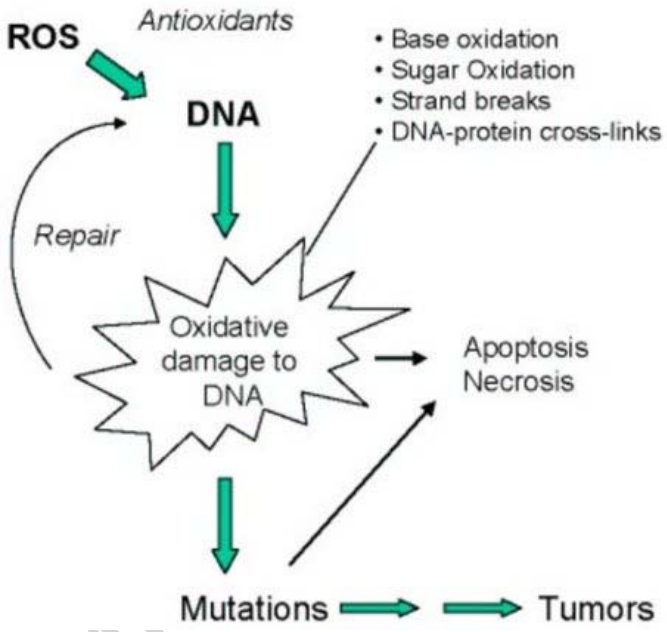

Figure 3. Oxidative damage can induce mutations and may lead to the formation of tumors.

Such genotoxic effects may be observed as cytogenetic measurements. These may include the induction of sister chromatid exchanges, chromosome aberrations or micronuclei. Sister chromatid exchanges are the reciprocal interchange of genetic material between sister chromatids on a chromosome; chromosome aberrations are abnormalities such as breaks or gaps in the chromosomes, which can be visualized by microscopical study of the chromosomes; and micronuclei are small masses of chromatin resulting from chromosomal breakages during cell division which can be found within the cytoplasm of cells in addition to the main nucleus. Figure 4 illustrates examples of (a) sister chromatid exchanges, (b) chromosome aberrations, and (c) micronucleus. Each of these genotoxic effects has been observed in the cells of organisms that have been exposed to metals and organic contaminants that interfere with the genetic material during mitosis. Such endpoints have therefore been applied as

general biomarkers of exposure to genotoxic agents. However, the endpoints are not contaminant- specific and may be induced by metals, organic or radioactive agents.

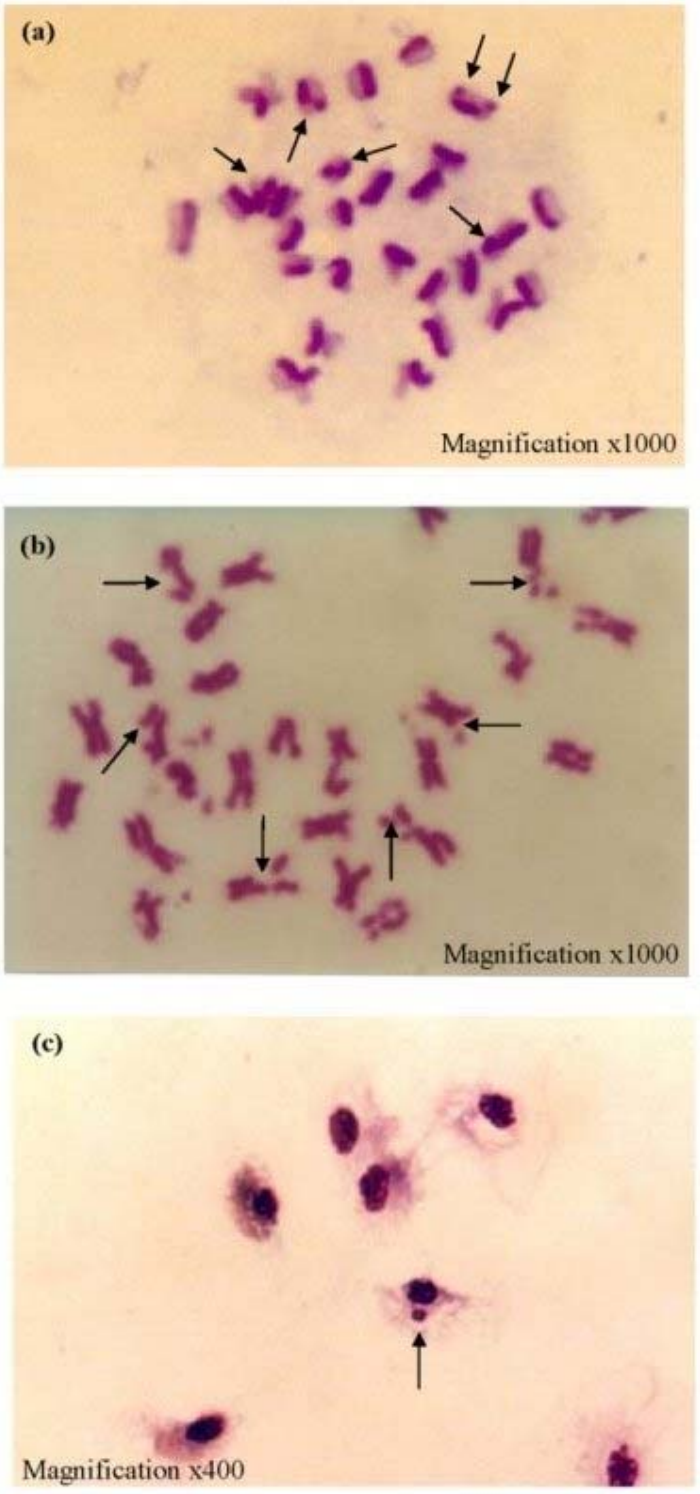

Figure 4. Examples of (a) sister chromatid exchanges, (b) chromosome aberrations, and (c) micronucleus in cells from the marine mussel *Mytilus edulis*.

To minimize the toxic potential of metals, many aquatic organisms sequester these contaminants. Metallothioneins (MTs) are widely distributed, low molecular weight proteins with a cysteine-rich polypeptide structure and high affinity for metals, suggesting an important role in the intracellular regulation and sequestering of these elements. Numerous authors have reported that functions attributed to MT include detoxification, storage and regulation of heavy metals. Previous authors have demonstrated the disruption of MT expression in oyster hemocytes prevents effective protection against cadmium toxicity. The induction of MT proteins in response to intracellular toxic metal concentrations forms the basis for application of this assay as a biomarker. However, it should also be considered that hormones, in particular progesterone and glucocorticoids might also have the potential to induce MT synthesis. Consequentially, differences may arise between species, reproductive condition, diet and season.

Specific studies where laboratory exposures of the mussel, *Mytilus edulis*, to the metal copper were undertaken, were reported to induce changes at the physiological level. In these studies, alterations in the behavior, respiration, filtration and ventilation activities were noted. These responses have been suggested as potential biomarkers of general stress, since they can be induced by contaminants other than heavy metals.

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Biographical Sketches

Victoria Cheung is a post-doctoral research fellow at the Plymouth Environmental Research Center, University of Plymouth. Her research interests are the application of biomarkers in aquatic invertebrates for the monitoring of the health status of the ecosystem. She is a member of the Society for Experimental Biology and the United Kingdom Mutagen Society.

Tamara Galloway is Principal Lecturer in Biochemistry at the Plymouth Environmental Research Center, University of Plymouth. Her research interests are in the adaptive biochemical mechanisms induced by chronic exposure to environmental contaminants and how these can be used as biomarkers of marine pollution in environmental monitoring. She is a committee member of the Biochemical Society and has been a workshop leader for United Nations Environment Programme, Global Investigation of Pollution in the Marine Environment (UNEP/GIPME) training programmes.

Michael Depledge is Head of Science for the Environment Agency, UK. He is an expert advisor to numerous governmental and international committees, notably the DEFRA Endocrine Disruption Expert Group, the Health of the Oceans Module (HOTO) of the Global Ocean Observation System (GOOS), United Nations Environment Programme and the International Oceanographic Commission/International Maritime Organisation/UNEP Scientific Advisory Group.