REVIEW
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ECOTOXICITY MONITORING – USE OF *Vibrio fischeri*

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The proliferation of chemical substances having the potential to pollute any environmental medium (air, land, water), or humans via occupational exposure, is considerable. Whilst chemical analytical techniques exist for the measurement of some of these chemicals, many of the methods involve costly techniques of considerable sophistication – quantification may be even more difficult. In less developed countries where sophisticated techniques may not be available or supplies of reagents, compressed gases or even electricity cannot be guaranteed, generic techniques have a great deal to offer. An emission of a chemical will cause adverse effects to organisms and hence there is an enormous advantage in measuring such effects on biological systems. One such technique is the reduction of light output in the presence of a toxicant to the marine bacteria *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum* NRRL B-11177). A dark variant M-169 can also be used to obtain mutagenicity data. A chronic test whose results compare well with *Ceriodaphnia dubia*, has also been developed. The development of the principles of environmental toxicology assessment is reviewed together with the concept of toxic insult as a pragmatic tool in environmental risk assessment.

Key terms:
chemical safety, environmental toxicology assessment, environmental xenobiotics, risk reduction

Environmental toxicology, unlike ecotoxicology, is used to study the direct effects of environmental chemicals on human beings. However, in view of the complexities of food chain effects, and as human beings are not isolated from the natural environment, effects of chemicals on ecosystems need to be considered. It should be remembered that human beings are an integral part of the natural environment, which they have modified by their activities.

The major problem associated with the measurement of organic substances is that no matter how sophisticated the analytical chemical technique used for the identification, and even less so for the quantification, of substances in a

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complex effluent stream, effluvia or leachate, only under the best of conditions is it possible to achieve ~20 per cent success. This is unlikely to include the enormous range of chemicals produced by chemical interaction, biological transformation, burning, etc. as a result of natural disasters or warfare.

Biological measurement techniques (i.e. biological markers) for environmental toxicology have been increasing in availability and scope over this decade. Their attraction is of obvious advantage and unlike chemical methods, they provide an indication of the toxicity of the sample investigated, e.g. its toxic insult (1–4).

THE MAGNITUDE OF THE PROBLEM

Both nature and chemists' activities have resulted in a high proliferation of chemical substances. Chemical Abstracts lists more than 12 million substances, the majority being natural in origin, that is, those synthesized by animals, bacteria, fish, microorganisms of all kinds, plants, etc. The range of organic chemical structures is also great, from very simple molecules such as methane to complex polypeptides, etc.

Chemists' activities have also been considerable during the past decade; biochemists in particular have mimicked nature, often utilizing bacterial and enzymic techniques to produce chemicals of equal complexity. Within the European Union more than 100,000 chemicals have been notified, and several thousands are being synthesized as chemicals of potential industrial importance every year.

The potential effects of some of these compounds on the natural environment are considerable. The pollution effects of heavy metals on the natural environment are very well documented. Some examples include:

- mercury from chloroalkali processes
- chromium from tanning
- PCBs, formerly used as dielectrics in transformers and condensers
- PAHs from fuels burnt at too low a temperature
- ethylene thiourea from semi-conductors causing inhibition of nitrification during sewage treatment.

Both natural and these xenobiotics are discharged to all environmental media – to air, water and to soil.

It is vital to remember that we are all dependent totally on chemicals:

- pharmaceuticals, for health care
- agrochemicals and pesticides to improve crop yields
- preservatives for food, paper, oils, etc.
- adhesives
- plastics
- colorants.

The list is almost endless. Hence, as there cannot be a return to the days of the hunter-gatherer, mankind has to know how to control and minimize these emissions.

It is essential to remember that emissions to air and water do not recognize national boundaries.

BIOLOGICAL MONITORING

Luminescence in *Vibrio fischeri*, formerly known as *Photobacterium phosphoreum* NRRL B-11177, is dependent upon both cell density and actively growing bacteria which undergo induction of the lux system during the late stage of exponential growth. This "auto-induction" phenomenon is attributed to the accumulation of a specific cell product, *N*-3-oxo-hexanoyl-*L*-homoserine lactone, in the culture medium (5–7).

Three types of assay procedures are now available:

Acute test – The Microtox® test

This well-known procedure is used globally to analyse many environmental samples, especially municipal and industrial wastes.

The Microtox test measures toxicity by measuring decreases in the light output of the bacteria. When properly maintained and grown, such bacteria divert less than 10 per cent of their metabolic energy into a special metabolic pathway that converts chemical energy, through the electron transport system, into visible light. The presence of toxic substances causes a disruption of this cellular metabolism and hence the light output.

Kaiser has reported on test data on more than 1000 organic chemicals. Results show a high collinearity between aquatic bioassays and Microtox data over a range of ~8 orders of magnitude (7, 8).

Genotoxicity measurements in environmental samples

A number of the methods available currently for estimating the genotoxicity of environmental samples have practical limitations. These include high cost, and complex protocols which require highly trained personnel to obtain reproducible test data.

The technology involved in the acute test (see above) has been extended to utilize a dark strain of these bacteria to detect the presence of genotoxicity in environmental samples (1, 3). This test – the Mutatox® test, uses a dark mutant (M-169) of *V. fischeri* that exhibits light production only when grown in the presence of sublethal concentrations of genotoxic agents. The results obtained have been compared with the Ames test and carcinogenicity data (6).

The Mutatox® test performed well when compared with other genotoxicity test results, e.g. Ames *Salmonella* tests, SOS chromotest, sister chromatid exchange, DNA alkaline elution tests, etc.

Development of the chronic test procedure

Only from a detailed understanding of the lux gene transcriptional control system was it possible to develop a chronic test system using *V. fischeri*. The test requirements included several cell divisions and complete induction of the luciferase system to provide adequate light to quantify the test endpoint. Initial evaluation of this chronic test method included a comparison with the *Ceriodaphnia dubia* chronic test (5).

There is excellent agreement between the two methods.

Biosensors

The development of biosensors, e.g. biological or molecular markers that can illustrate a measurable effect in a biological medium, tissues, cells or fluids, has to be one of the key developmental tasks for the end of the 20th century. Such markers will develop the concept of toxic insult without the precise chemical knowledge of the toxic agent. This in turn leads to a marker indicating a biologically effective dose, i.e. the amount of absorbed chemical that has interacted with critical subcellular targets, measured in either a target or surrogate tissue. Hence, sequentially, exposure will lead to: internal dose → biologically effective dose → biological response → altered structure/function → disease → prognosis.

These stages will take into account susceptibility and an environmental lifestyle.

Through the development of biosensors or biomarkers and their associated molecular and toxicological techniques, an understanding of the intermediate steps between exposure and disease occurrence can improve the precision of the mode of exposure and outcome measurements. If epidemiological techniques are also deployed, as they include large numbers of participants, the biological markers that are most desirable for use in human studies are those that may be measured with a minimum skill or equipment requirement in the smallest quantity of biological media and can be obtained with minimal invasive techniques. Additionally, these should be inexpensive, sensitive, as specific as possible, robust, peer reviewed and reliable. For such research and collaboration to succeed, researchers from many disciplines must learn the strength, weakness and, above all, the "language" of other disciplines. Only by such undertakings can environmental toxicology assessments be successful.

Environmental risk

The estimation of the risk to human health from both xenobiotic and natural substances within our society has become increasingly critical. Toxic effects to both animals and other species are undertaken experimentally at high doses, usually for short periods. However, low-level chronic exposure effects largely remain unknown or could be biological, e.g. induction of cytochrome enzymes. Whilst for the short-term administration of drugs this is acceptable, it is not so for chronic exposure to environmental pollutants. Furthermore, ecotoxicological effects to individuals are often of less importance than are the effects to whole ecosystems and to populations. Reliance on extrapolation from animal studies can, and often has, led to a serious underestimation or overestimation of the risk.

Regulations and legislation based on overestimates can have serious and unnecessary economic consequences by keeping economically desirable products from being continued to be marketed and used to enact clean-up operations which have little real benefit. No action can equally be disastrous and many examples of both laissez-faire policies, often resulting from political dogma, are well documented in Central and Eastern Europe and other developing countries.

Properly conducted animal experiments and epidemiological studies provide a basis for the linkage between human health risks and environmental factors; from such associations, public health and environmental protection policies can be developed so as to minimize the risks to current and future populations.

RISK REDUCTION

Techniques in risk reduction are now well documented (9). Risk reduction is a very necessary requirement and follows readily from undertaking a risk management. It entails adding value to data and information in a ready to use form for use by policy makers, planners, developers and reformers to guide measured and programme agendas for both environmental and occupational exposure, care and protection. For the natural environment due care will need to be addressed to the presence of all environmental xenobiotics (10).

THE FUTURE

Chemicals are essential to mankind. Chemical safety is of paramount importance. For the future, and especially in developing countries, the use of generic techniques particularly those using enzyme systems, e.g. luciferase, cytochrome P-450, EROD, umu C-assays, etc., have to be the way forward. However, in order to reduce pollution, it is incumbent upon the chemical industry, governments, and in particular end-users, including the public, to improve the care in the management and wastage of chemicals. This can be accomplished, for example by the use of granulation for agrochemicals, release formulations, water-based paints, chromium alternatives in tanning, manufacture at the point of use, thus reducing transport, use of environmentally more friendly chemicals, and use of clean technologies.

Synthesis techniques, such as flash reactors in 2 L reactors instead of stirred 10,000 L vessels, result in negligible production of highly hazardous waste products and decrease accidents. The knowledge of toxicology, and above all ecotoxicology, within developing countries will thus result in risk reduction becoming a reality. This means waste reduction, increased recycling, and the use of waste materials, less transport over hazardous terrain of hazardous intermediates. Greater appreciation, training and international recognition of exchangeable and able to be registered, and peer reviewed/assessed qualifications by scientists are of increasing necessity. Politicians also need to get together with the media so as to appreciate the real risks associated with the use of chemicals on which we now all depend.

One of the most important requirements in developing countries is the promulgation of a sound environmental protection act and its enactment through an environmental protection agency. This should be drawn up with liaison between government officials, trade associations, chambers of commerce, and industry. The act must take into account integrated pollution control, best practical environmental options and best available techniques but not entailing excessive costs. It is important to stress the need to apply techniques, e.g. management training, supervision and qualifications of staff including an adequate knowledge base in ecotoxicology, environmental toxicology assessment, risk reduction, leading to chemical safety.

One of the greatest problems, as we near the end of the 20th century is the availability of pure and wholesome water supplies uncontaminated with microorganisms. Fortunately, this can now be achieved by use of *in situ* electrogeneration of chlorine.

Such legislation must be enforceable, controllable and pragmatic, without encumbering industry with excessive fees or fines. Monitoring of environmental parameters need to be stipulated embodying measurements that are within the infrastructural abilities of a country's resources or world aid donations. For this reason, generic methods, at least initially, need to be given high priority.

Centres of expertise in ecotoxicology, environmental monitoring, environmental toxicology assessment, risk reduction, leading to chemical safety, need to be established. Regional centres of excellence can then be set up to service the most sophisticated requirements within the region.

Everyone should be aware that pollution is the responsibility for themselves, their offspring, and their neighbors. Neither poisonous gas clouds nor pollutants in rivers or oceans recognize national boundaries. Crops grown on contaminated land, or dairy products, etc., are often transported thousands of kilometres for sale in supermarkets on a global basis. Hence, the message for the future has to be: "Think globally: act locally".

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Sažetak

PRAĆENJE EKOTOKSIČNOSTI – UPORABA BAKTERIJE *Vibrio fischeri*

Kemijske tvari koje su u stanju onečistiti svaki medij u okolišu (zrak, zemlju, vodu), odnosno utjecati na ljude koji su im profesionalno izloženi vrlo su rasprostranjene. Chemical Abstracts navodi više od 12 milijuna supstancija a više od 100.000 zabilježeno je u industrijskoj uporabi Europske unije. Premda za mjerenje nekih takvih kemijskih spojeva postoje kemijski analitički postupci – mnoge metode uključuju skupe i znatno usavršene postupke – čini se da su kvantitativna mjerenja izravnije primijenjena. Dok se podaci o rizičnosti, odnosno pouzdani toksikološki i ekotoksikološki podaci potvrđuju testovima na sisavcima, ribama i drugim organizmima, često nedostaje podataka o mnogim kemijskim spojevima. Podaci o mnogim prirodnim proizvodima, čija toksičnost katkad nadilazi toksičnost bilo kojeg industrijskog kemijskog spoja, o metabolitima u otpadnim vodama, o nusproizvodima izgaranja izazvanih bilo prirodnim procesima (vulkani) ili uzrokovanih čovjekom (ratovi) nedostatno su dokumentirani za svaki spoj ili smjesu posebno. U manje razvijenim zemljama kojima nedostaju sofisticirani postupci, odnosno zalihe reagensa, komprimiranih plinova pa čak i opskrba električnom energijom nije pouzdana, osnovni postupci jesu metode izbora. Ispuštanje kemijskih spojeva u okoliš štetno utječe na organizme pa je stoga mjerenje takvih učinaka na biosisteme vrlo korisno. Jedan se takav postupak temelji na smanjenju svjetlosne emisije kod morske bakterije *Vibrio fischeri* (prethodno poznate kao *Photobacterium phosphoreum* NRRL B-11177) u prisutnosti toksične tvari. Nesvjetleća varijanta bakterije, M-169, također se može upotrijebiti za dobivanje podataka o mutagenosti. Također je razvijeno i kronično testiranje rezultati kojega se mogu dobro usporediti s *Ceriodaphnia dubia.* Ovaj rad raspravlja o navedenim postupcima te daje pregled razvoja načela toksikološke procjene okoliša uključivši i koncept toksičnog oštećenja kao korisnog pomagala pri procjeni ugroženosti okoliša.

Ključne riječi:
kemijska sigurnost, ksenobiotici u okolišu, smanjenje rizika, toksikološka procjena okoliša

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