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S.S. Rao and D.A. Rokosh

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[1]S.S. Rao and [2]D.A. Rokosh

[1]Aquatic Ecosystem Conservation Branch
National Water Research Institute
Environment Canada
867 Lakeshore Road, P.O. Box 5050
Burlington, Ontario L7R 4A6

[2]392 Swanson Court
Burlington, Ontario
L7R 4G6

NWRI Contribution No. 00-151

MANAGEMENT PERSPECTIVE

Environmental Effects Monitoring (EEM) continues to be an important focus of NWRI's research since studies of this kind address important ecological issues and fall under the overall thrust of Environment Canada's research program. Application of tools assessing the impact of environmental contaminants on aquatic biota has continued to be a major focus of NWRI's Ecosystem Health Assessment research. An important NWRI initiative has been the development of techniques to identify genotoxic contaminants with the potential to cause genetic damage in aquatic organisms.

Earlier work at NWRI has led to the development of practical environmental effects monitoring tools applicable to the assessment of the impact of environmental toxicants and genotoxicants and as such served as a practical approach to aid in the achievement of Environment Canada's EEM objectives. However, use of such techniques to evaluate the impact of genotoxic contaminants on aquatic organisms remain in its infancy.

This article highlights some important research developments from the last decade which may make investigations of health implications of environmental genotoxins more fruitful. We also review the potential uses of monitoring tools for the assessment of environmental genotoxins on biota and suggest strategies to be applied in future studies of the impact of genotoxic contaminants on ecosystem health.

SOMMAIRE À L'INTENTION DE LA DIRECTION

La surveillance des effets environnementaux continue d'être un domaine de recherche important pour l'INRE puisque les études de ce genre abordent des problèmes écologiques importants et font partie de l'objectif général du programme de recherche d'Environnement Canada. L'application d'instruments d'évaluation de l'impact des contaminants environnementaux sur le biote aquatique reste une priorité importante de la recherche en matière d'évaluation de l'état des écosystèmes. Une initiative importante de l'INRE a consisté en l'élaboration de techniques permettant d'identifier les contaminants génotoxiques qui sont susceptibles de causer des dommages génétiques aux organismes aquatiques.

Des travaux antérieurs effectués à l'INRE avaient amené l'élaboration d'instruments pratiques de surveillance des incidences environnementales applicables à l'évaluation de l'impact des substances toxiques pour l'environnement et des substances génotoxiques, ce qui a constitué une approche pratique au service de la réalisation de l'objectif de surveillance des effets environnementaux d'Environnement Canada. Toutefois, l'utilisation de ces techniques en vue d'évaluer l'impact des contaminants génotoxiques sur les organismes aquatiques en est encore aux premiers balbutiements.

Cet article souligne quelques importantes percées en recherche effectuées au cours de la dernière décennie et qui pourraient rendre plus productives les recherches sur les implications sanitaires des génotoxines environnementales. Nous examinons également les utilisations potentielles des instruments de surveillance pour l'évaluation de l'effet des génotoxines sur le biote et suggérons des stratégies à appliquer au cours des futures études concernant l'impact des contaminants sur l'état de l'écosystème.

ABSTRACT

Application of tools for assessing the impact of environmental contaminants on aquatic biota has continued to be a major focus of NWRI's Ecosystem Health Assessment research. An important NWRI initiative has been the development of techniques to identify genotoxic contaminants with the potential to cause genetic damage in aquatic organisms. However, use of such techniques to evaluate the impact of genotoxic contaminants on aquatic organisms remains in its infancy. This article highlights some important research developments from the last decade which may make investigations of health implications of environmental genotoxins more fruitful. We also review potential use of monitoring tools for the assessment of environmental genotoxins on the biota and suggest strategies to be applied in future studies of the impact of genotoxic contaminants on ecosystem health.

Keywords: environmental genotoxins, ecosystem health, DNA damage, genotoxic endpoint, genetic diversity, aquatic biota, bioassays

RÉSUMÉ

L'application d'instruments d'évaluation de l'impact des contaminants environnementaux sur le biote aquatique a continué d'occuper une place centrale dans la recherche de l'INRE en matière d'évaluation de l'état de l'écosystème. Une initiative importante de l'INRE a consisté en l'élaboration de techniques permettant d'identifier les contaminants génotoxiques qui sont susceptibles de causer des dommages génétiques aux organismes aquatiques. Toutefois, l'utilisation de ces techniques en vue d'évaluer l'impact des contaminants génotoxiques sur les organismes aquatiques en est encore aux premiers balbutiements. Cet article souligne quelques importantes percées en recherche effectuées au cours de la dernière décennie et qui pourraient rendre plus productives les recherches sur les implications sanitaires des génotoxines environnementales. Nous examinons également les utilisations potentielles des instruments de surveillance pour l'évaluation de l'effet des génotoxines sur le biote et suggérons des stratégies à appliquer au cours des futures études concernant l'impact des contaminants sur l'état de l'écosystème.

Mots clés : génotoxines environnementales, état de l'écosystème, altération de l'ADN, résultats génotoxiques, diversité génétique, biote aquatique, essais biologiques.

INTRODUCTION

Genotoxic contaminants are an environmental concern because of their potential to cause a variety of diseases including heritable birth defects, premature aging, immunological diseases and cancer. Historically, considerable effort has been applied to the identification of genotoxic substances and evaluation of their health risk, principally directed at the protection of human health. Genotoxic contaminants potentially effect all organisms. A recent report by Anderson et al. (1998) describes the state-of-the-art in research of genotoxic damage to environmental organisms. In addition to an evaluation of monitoring tools and their application in environmental studies, the report suggests future research applicable to the Canadian environment.

The increasingly complex nature of industrial pollutants, discharged into our aquatic environment, have stimulated development of new directions in environmental monitoring programs. While measurement of pollutant toxicity has long been established in environmental monitoring programs, the assessment of more subtle effects such as genotoxicity remains in it's infancy. In reality, damage to the genetic integrity of individuals may have a much wider and irreversible impact on the health of environmental organisms The recent science of eco-genotoxicology addresses both the toxicity of genotoxic substances and their potential impact on ecosystem health. Quantifying ecotoxicological effects of pollutants is, in fact, critical to the protection of aquatic ecosystem health (Birge et al., 1989).

Between 1981 and 1985 the International Program on Chemical Safety (IPCS) completed a large international collaborative study of both *in vitro* and *in vivo* short term assays used to identify chemical mutagens and carcinogens. In the main such assays are designed to assess the toxicity of chemicals as they relate to human health. Several health regulatory agencies use the results of genotoxicity assays as a critical component in the assessment of human health risk of new and existing commercial products.

Over the last 10 years, there has been a significant increase in the application of genotoxicity tests in field investigations (Shugart et al., 1992; Wurgler and Kramers, 1992, Herbert and Luiker, 1996.) These studies have demonstrated the potential utility of existing techniques in assessing a range of compelling environmental issues. It is valuable to use genotoxic bio-markers, because they can provide information on the potential impacts on the health of populations of environmental organisms. Furthermore, the increasing power of these genetic toxicology techniques has created new possibilities for detecting both direct and indirect effects of contaminants on genetic patterns of these populations. Moreover, these bio-markers can be linked at times with processes of cellular damage leading to reduced performance of individuals as well as the reproductive success of the population as a whole.

The recent (1998) 8th annual meeting of SETAC-Europe included a session on eco-genotoxicology where genetic effects in the aquatic environment were discussed. Particular topics addressed were the regulatory aspects of genotoxicology as well as the inadequacy or lack of knowledge of repair of DNA damage in aquatic biota.

The potential course of future environmental studies is outlined in a report jointly submitted to the Atomic Energy Control Board and Environment Canada by Anderson et al.,(1998) which describes current research and makes suggestions for future directions. That report outlines bioassays used in the identification of genotoxicity in environmental discharges and tools used to identify impacts of genotoxic substances on aquatic organisms. That report also suggests strategies that may be applied to demonstrate impacts of genotoxic pollutants on the health of individual and populations of environmental organisms. Lastly Anderson et al. identify in their report study areas in Canada where genotoxicity studies may be applied.

The present document complements the work of Anderson et al. (1998) by summarizing their conclusions in the light of more recent research developments and by considering some of their recommendations within the context of Environment Canada's research needs.

Environmental Genotoxins - A Toxicological End Point

The purpose of toxicity testing is to monitor and predict the effects of pollutants on the long-term health of populations, communities and ecosystems (Giesy and Garney, 1989), however, the laboratory tests to predict effects in the field have been critisized (Cairns, 1983; 1986; Odum, 1984: Barbour et al., 1996), because these tests or approaches measure only direct toxic effects on the test organisms. Furthermore, under controlled laboratory conditions, biological and chemical interactions are minimized, exposure concentrations are held constant, and important ecosystem functions such as energy flow are not considered (Adams et. al., 1983)

A strategy for the assessment of the effects of genotoxic pollutants on the health of environmental organisms should include three critical components; detection and quantitation of sources of genotoxic pollutants, demonstration of an impact of these pollutants on environmental organisms and determination that these effects have a detrimental effect on environmental populations.

1. Detection and Quantitation of Genotoxic Discharges

Studies to measure the presence and intensity of genotoxic activity of substances discharged into the environment.

The analytical tools necessary to address this issue exist and can be readily applied in monitoring programs. Chemical quantitation and characterization techniques can measure individual chemical compounds and predict the genotoxic potential of individual pollutants. Physical methods are available to measure the radioactivity of environmental discharges. Laboratory based bioassays, such as the Mutagenicity test and many others, are available to measure the genotoxic potential of individual compounds, chemical mixtures, environmental samples and sample extracts. For example, the SOS chromotest, particularly the semi-automated microplate version described by White et al., (1996) can be used in testing of environmental samples permitting the general assessment of sources and ecological behavior of genotoxic organic contaminants in aquatic systems. This assay is well suited to subsequent bioassay-directed chemical analysis. However, if the genotoxic potential of radioactive discharges are assessed, bioassays specific to these genotoxic agents may have to be further developed.

Studies characterizing environmental sources of genotoxicity are feasible and can be readily applied. Results of such studies may be used in prioritizing areas of concern. Results of individual tests may be considered in defending concerns of likely environmental impairment.

However such laboratory based chemical, physical or biological tests have one serious flaw. Such tests are not suitable scientifically in demonstration of a genotoxic impact on individuals or populations of environmental organisms.

2. Measurement of genotoxic impacts in environmental organisms

Studies to measure genetic damage in organisms.

Historically and recently developed biological tests are potentially available to demonstrate genetic damage in environmental organisms. Signs of genetic damage, including primary DNA damage (by DNA breaks or DNA fragmentation), chromosomal damage (by sister chromatid exchanges, micro nuclei, altered chromosomal patterns or nuclear size variation), heritable mutation (by genetic markers) or carcinogenicity (by increased cancer incidence) may be measured. Application of cytogenetic tests for DNA and chromosomal damage have been demonstrated for aquatic organisms such as fish. Application of mutational assays have been historically demonstrated in plant and insect systems. Carcinogenicity bioassays, historically applied in mammalian (rodent) systems, have also been applied to aquatic (fish) organisms in laboratory or field studies. With recent developments, the application of a variety of bioassays demonstrating genetic damage in environmental organisms may be seriously considered in monitoring programs.

Assessment of the effects of genotoxic substances in aquatic organisms have been done in a variety of tests. Essentially, the nuclear damage in aquatic biota due to environmental genotoxins is considered as a first signal. Indicators of genotoxic effects including DNA strand breaks, sister chromatid exchanges, micro nuclei, anaphase and metaphase aberrations, DNA content by flow cytometry, mutations and DNA-Protein cross-links have been used to identify genotoxic effects in some natural organisms.

As for *in vivo* assays, there are limited options for assays related to assessing genotoxic endpoints with aquatic organisms (e.g. fish). Anaphase cytogenetics, because of the lack of high mitotic index in adult tissues, has severe limitations for its applications in field studies. Similarly, metaphase cytogenetic (sister chromosome exchange) are time consuming and require considerable initial method development. They are therefore not recommended for routine monitoring studies. The Comet Assay is one possibility, although much more work has to be done to validate this assay for both field monitoring and laboratory testing. The micro nucleus assay is time-consuming, and possibly subject to false positives in inexperienced hands. The 32P- post labeling technique for DNA adducts appears very sensitive for monitoring genotoxicity in fish exposed to aromatic compounds , but this technique is technically demanding and expensive. The UDS assay and DNA-unwinding assays also appear to suffer from a lack of sensitivity (especially for aromatic compounds, such as PAHs) and are technically-demanding protocols. Sister chromatid exchange assays and other metaphase techniques are not useful for most fish species that have large numbers of small chromosomes.

Although feasible, considerable research and development will still be required before the extent of genotoxic damage in individual environmental organisms can be truly assessed. Initially, suitable indicator species must be selected with the ability to detect an effect from a variety of potential genotoxic insults. In selection of indicator species, development should consider the atmospheric and terrestrial environment, as well as the aquatic environment, as a potential source of genotoxic pollutants. In selection of signs of genetic damage, priority should be given to end-points reflecting irreparable, heritable or debilitating effects. Studies should be conducted measuring natural levels of genetic damage, as a basis for demonstrating a significant increase in damage at affected areas. Lastly, it would be desirable to develop chemical-biological tools linking genetic damage in organisms with specific pollutants and sources of genotoxic contaminants.

Studies measuring genetic damage in individual environmental organisms are feasible. However, measurement of increased damage in individual organisms should, at best, be considered as the first step on the impact of ecosystem health. At this stage, effects are measured in individuals of selected environmental species. Measurement of a genetic effect at the population level of one or more species should be considered the definitive sign of impairment of ecosystem health.

3. Demonstration of a genetically related impact on the health of individual or populations of environmental organisms.

Studies to measure a change in the genetic integrity of populations of organisms.

The definitive study demonstrates this change may compromise population survival or result in the heritable transfer of mutations to offspring.

Biological tools measuring genetic characteristics of species of populations are rare with likely none currently suitable for environmental monitoring programs. Historically, biological tests using insect (Drosophila -fruit flies), plant (eg. barley) and perhaps fish (eg. Salmonoids) have been developed. These tests were used in measuring the genetic composition of populations or change in composition resulting from exposure to mutagenic agents. Their suitability is in the availability of a range of genetic markers which can readily be measured in populations. When employed in multiple generation studies, the competitive advantage of a particular genetic change or shifts in population genetic equilibria can also be measured.

Considerable research would be required to develop similar biological tools suitable for environmental studies. On the immediate horizon, bioassays employing plant populations may be most readily developed. With any system considerable research would be required in development of genetic markers. Once developed, multiple generation studies would require considerable time and resources. Nevertheless, studies of this type will be required to conclusively demonstrate an effect by genotoxic pollutants on the health of populations of environmental organisms.

Environment Canada's Role in the Development, Standardization and Technology Transfer of Genotoxicity Bioassay Applicable to Ecosystem Health Assessment Studies.

Currently many approaches are used to assess the impacts of these complex contaminants. In the past NWRI routinely conducted genotoxicity tests on environmental samples and aquatic biota. These assays include the Ames test, SOS Chromotest, Mutachromoplate Mutagenicity test, Micro nucleus Assay among others. However, some of these assays may have some limitations as indicated above.

Future efforts if directed towards the development and standardization of the following Genotoxicity technology will not only enhances the leadership and visibility of NWRI, but will help other organizations and universities to adopt the standardized technology in their environmental genotoxicity research studies.

Researchers at NWRI have developed and standardized a simplified mutagenicity technology (Mutachromoplate Mutagenicity) designed to detect the impact of mutagenic substances in environmental samples (Rao and Lifshitz, 1995). This technology has been widely used in University and Institutions in Canada and in developing countries.

In future, NWRI could take a lead role in the design of strategies and the development and application of specific technologies assessing impacts of genotoxic pollutants on the health of environmental organisms.

1. NWRI scientist should take a leading role in development of strategies to identify sources of genotoxic pollutants and to scientifically determine impacts of these pollutants on the health of individual and populations of environmental organisms.

2. NWRI should retain the chemical and biological techniques to measure the genotoxic potential in environmental pollution sources.

3. NWRI should enter into partnership efforts to develop indicator species applied in the measurement of individual and population level impacts of genotoxic pollutants on environmental organisms. Initial efforts should emphasize aquatic organisms but future development should consider organisms effected by the atmospheric and terrestrial environments

4. NWRI should enter into partnership efforts to develop genotoxicity bio-markers for genotoxic effects in environmental organisms. At this time, three technologies could be considered

    a. Flow Cytometry may be an extremely useful tool in the monitoring and detection of genetic (clastogenic) damage in both laboratory and field applications (Geay et al., 1979; Bickham et al.,1992; Lamb et al., 1991; Custer et al., 1994). This versatile tool can be used to assess DNA content variation in a variety of test organisms and tissues. Preliminary field studies have demonstrated increased variations, as well as dose and time dependent responses, in DNA content of exposed environmental populations. More widespread application of this technique is warranted since variations in the DNA content of blood cells may indicate a genotoxic impact of environmental contaminants and hence a potential impact on ecosystem health.

b. The validation of the Comet Assay for both field monitoring and laboratory testing to detect genetic damage in fish is essential and should involve initial comparisons with other genotoxicity techniques. The Comet Assay which gives a measure of genetic damage in the form of breaks in the DNA strands may also indicate potential ecosystem health damage

c. New advances in analytical instrumentation open up the possibility of monitoring for DNA adducts in fish exposed to some aromatic compounds for genotoxicity without radioactive post-labeling techniques. For instance, the capillary electrophoresis or liquid chromatography separation techniques coupled with Time-of-Flight mass spectrometry (mass range up to 12 kDa) are well suited to direct DNA adduct This assay may provide a tool to link genotoxic damage with environmental sources of genotoxic pollutants.

5. NWRI should enter into partnerships to develop genetic tests measuring genetic equilibria and changes in genetic composition in laboratory and environmental populations of indicator species.

In conclusion we recommend that the above genotoxicity technology be used in Ecosystem Health Studies to evaluate the implications of environmental genotoxins on aquatic biota. More specifically, Flow Cytometry, the Comet Assay and DNA adduct are of potential value in the studies within the Ecosystem Health Assessment project .

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867 Lakeshore Road
Burlington, Ontario
Canada L7R 4A6

Institut national de recherche sur les eaux
Environnement Canada
Centre canadien des eaux intérieures
Case postale 5050
867, chemin Lakeshore
Burlington, (Ontario)
Canada L7R 4A6

National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
Canada S7N 3H5

Centre national de recherche en hydrologie
11, boulevard Innovation
Saskatoon, (Saskatchewan)
Canada S7N 3H5

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