
Environmental Genotoxicity: Probing the Underlying Mechanisms

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Environmental pollution is a complex issue because of the diversity of anthropogenic agents, both chemical and physical, that have been detected and catalogued. The consequences to biota from exposure to genotoxic agents present an additional problem because of the potential for these agents to produce adverse change at the cellular and organismal levels. Past studies in genetic toxicology at the Oak Ridge National Laboratory have focused on structural damage to the DNA of environmental species that may occur after exposure to genotoxic agents and the use of this information to document exposure and to monitor remediation. In an effort to predict effects at the population, community, and ecosystem levels, current studies in genetic ecotoxicology are attempting to characterize the biologic mechanisms at the gene level that regulate and limit the response of an individual organism to genotoxic factors in their environment. — *Environ Health Perspect* 102(Suppl 12):13–17 (1994)

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Introduction

Pollution of the environment has become a major concern of society. Perhaps one of the more serious concerns is the potential for exposure to substances that are genotoxic. This problem arises because some of these pollutants are carcinogens and mutagens with the capacity to affect both the structural integrity of DNA and the fidelity of its biologic expression (1).

Genetic toxicology is an area of science in which the interaction of DNA-damaging agents with the cell's genetic material is studied in relation to subsequent effect(s) on the health of the organism. Structural changes to the integrity of DNA caused by DNA-damaging agents are useful end points for assessing exposure to hazardous environmental pollutants on human health (2,3) and biota (4,5). The organism functions as an integrator of exposure, accounting for abiotic and physiologic factors that modulate the dose of toxicant taken up, and the resulting magnitude of the change in DNA structure provides an estimate of the severity of exposure, hopefully in time to take preventive or remedial measures.

Genetic ecotoxicology is an approach that applies the principles and techniques of genetic toxicology to assess the potential effects of environmental pollution, in the form of genotoxic agents, on the health of the ecosystem. To this end, recent advances in toxicology, clinical medicine, and molecular genetics will foster a better understanding of the biological, chemical, and physical processes that accompany exposure to genotoxic agents. Because the techniques and methods unique to these disciplines are extremely sensitive and specific, it is anticipated that their implementation into studies concerned with the mechanism of action of genotoxicants will provide a stronger scientific basis for the assessment of risk of exposure.

For these and other reasons, the Biological Markers Group in the Environmental Sciences Division at the Oak Ridge National Laboratory (ORNL) has included genotoxicity studies as part of its activities concerned with the biologic monitoring of environmental pollution. The remainder of this report will provide examples of problems concerning genotoxic agents in the environment and the approaches/techniques used to address these problems. In most instances our studies have been concerned with documenting exposure of environmental species to genotoxic agents via the detection of DNA structural damage. DNA was analyzed for specific modifications such as chemical adducts (covalent attachment of a specific chemical to DNA) and photoproducts (dimerization of bases due to ultraviolet light) or generalized structural damage (i.e., DNA strand breakage) that is induced from exposure to any of a number of genotoxi-

cants. Each example contains a brief description about the environmental issue or concern being addressed, the approach used (i.e., species sampled and methodology employed to detect DNA damage), and results obtained. Finally, in an effort to define the potential consequence of exposure to genotoxicants at organizational levels beyond the individual, two new approaches are described that utilize current techniques of molecular biology.

DNA Adducts in Beluga Whales

Exposure of an organism to a genotoxic chemical may result in the formation of a covalently attached intermediate to the organism's DNA (adduct). Thus, detection of adducts provides a way of documenting exposure. This approach was used to examine DNA from beluga whales of the St. Lawrence estuary to determine whether exposure to benzo[*a*]pyrene (BaP), a potent environmental carcinogen and the suspected etiologic agent for the high incidence of cancer in these animals (6), had occurred. Data on BaP adducts (7) in the DNA of brain tissue from stranded beluga whales from the St. Lawrence estuary and in the DNA of brain and liver tissues from whales from the Mackenzie Estuary are shown in Table 1. Detection of BaP adducts of the whale DNA was by HPLC/fluorescence analysis (8), a technique that measures only adducts that form between the DNA and the ultimate carcinogenic form of BaP. Values obtained from the St. Lawrence belugas approach those found in animals, both terrestrial and aquatic, exposed under laboratory conditions to carcinogenic

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Table 1. Detection of benzo[a]pyrene adducts in DNA of beluga whales.

Sample	Tissue	BaP adduct formation
St. Lawrence Estuary		
#1	Brain	206
#2	Brain	94
#3	Brain	69
Mackenzie Estuary		
#1-#4	Brain	ND
#1-#4	Liver	ND

ND, none detected. Analyses for BaP adducts to DNA were as described by Shugart et al. (8), and data expressed as nanograms of BaP tetrol I-1 per gram of DNA.

doses of BaP. No detectable adducts were noted in the DNA of whales from the Mackenzie Estuary.

DNA Strand Breaks in Turtles and Sunfish

Exposure to genotoxic agents may cause, in addition to or concomitant with adduct formation, other types of damage to the DNA molecule. Strand breakage in the DNA molecule occurs under normal conditions but exposure to genotoxicants can increase the amount. Recent reports (4,9) have detailed the various types of structural changes that may occur to DNA under normal cellular conditions as well as after exposure to chemical and physical genotoxicants that may potentiate strand breakage. For example, ionizing radiation can cause strand breakage directly, whereas other physical agents such as UV light or genotoxic chemicals can cause alterations to the DNA molecules that are candidates for repair (e.g., photoproducts, adducts, modified bases, etc.) and thus for the occurrence of strand breaks (9).

Early in 1987, the detection of excessive strand breakage in the DNA of several aquatic species was implemented as a biologic monitor for environmental genotoxicity as a part of the Biological Monitoring and Abatement Program for the U.S. Department of Energy (USDOE) Reservation in Oak Ridge, Tennessee. DNA strand breakage as an end point of genotoxicant insult was used for two important reasons. First, it is compatible with routine monitoring because the analysis (alkaline unwinding assay) for this type of damage is easy to perform (10) and cost effective; second, the assay provides a measure of DNA strand breaks arising from several contaminant-mediated processes (9). Examples with two different aquatic species will suffice to demonstrate the suitability of the approach.

Two species of turtles, the common snapping turtle (*Chelydra serpentina*) and the pond slider (*Trachemys scripta*) were compared for their usefulness as biologic sentinels for environmental genotoxicants in White Oak Lake on the USDOE Reservation (11). White Oak Lake is a settling basin for low-level radioactive and nonradioactive wastes generated at ORNL since 1943. It supports a high diversity of turtle species, with *T. scripta* the most abundant and *C. serpentina* as the second most abundant. Cesium-137, cobalt-60, strontium-90, and tritium contribute most of the radioactivity to the lake. Species-specific data collected on DNA strand breakage in turtles captured in White Oak Lake were compared to data from the Bearden Creek embayment, a reference site with similar biota but with no known history of contamination by hazardous chemicals. Over the entire course of the study, genotoxic stress was evident in both species taken from White Oak Lake. This is graphically represented in Figure 1, in which individual F values are plotted in relation to when and where the turtles were captured. F values are a measure of the relative double-strandedness of a particular DNA preparation which in turn can be related to the number of strand breaks present. F values are determined

under *in vitro* conditions by the alkaline unwinding assay (10) where the rate of conversion of the DNA from double-stranded to single-stranded structures is proportional to the number of strand breaks present. Thus large F values are indicative of DNA with few strand breaks. The F values for both species of turtles reveal a significant ($p < 0.001$) site effect and indicate that the DNA in these species have higher levels of strand breaks than the same species from the reference site. It should be noted that Lamb et al. (12) also detected DNA damage by flow cytometric analysis in turtles occupying seepage basins containing radioactive contaminants.

Analyzing for strand breaks in the DNA of sunfish has been employed as a biologic marker for environmental genotoxicity as part of the Biological Monitoring and Abatement Program at East Fork Poplar Creek (13). This creek is the receiving stream for industrial effluent from the USDOE reservation in Oak Ridge, TN. Water and sediments downstream contain metals, organic chemicals, and radionuclides discharged over many years of operation (13).

DNA strand break data (F values), measured in sunfish from the head waters of the creek (near the USDOE reservation) and at Hinds Creek (reference stream) over

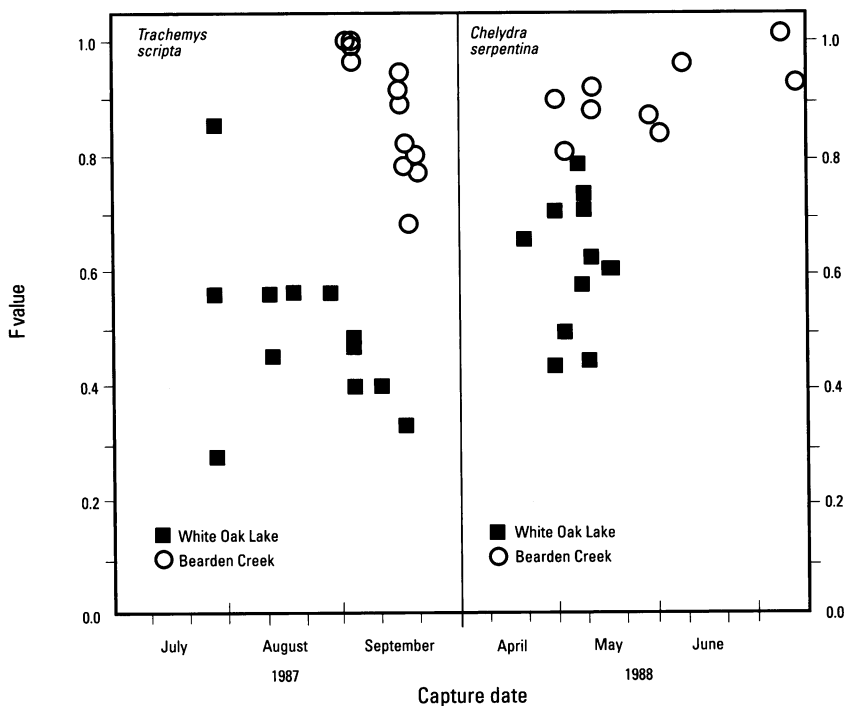


Figure 1. Fraction of double-stranded (F value) DNA in liver samples of *Trachemys scripta* and *Chelydra serpentina* collected from the Oak Ridge Reservation [from Shugart (10), with permission].

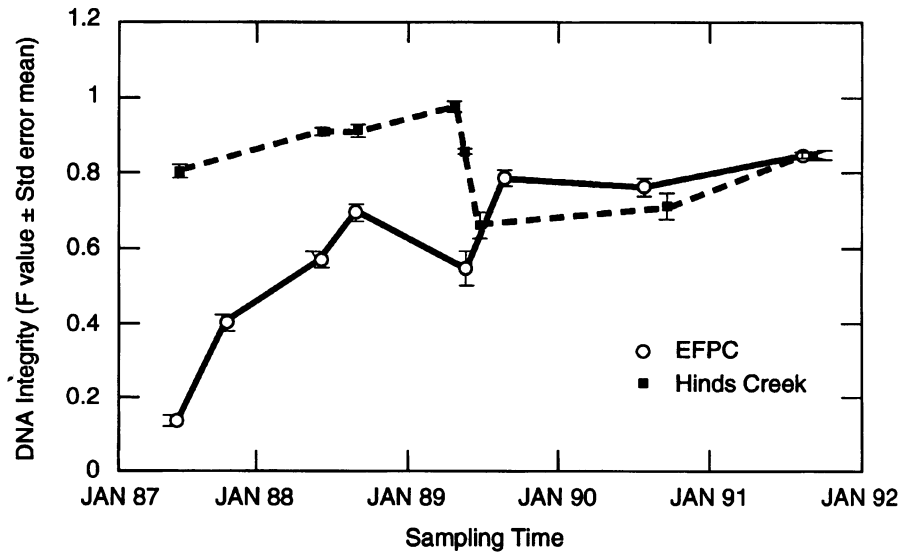


Figure 2. Temporal status of double-stranded (F value) DNA in liver samples of sunfish from East Fork Poplar Creek (contaminated stream) and Hinds Creek (reference stream) over a 4-year period.

a period of 4 years are presented in Figure 2. Two points are clear: that DNA structural integrity of the sunfish from the reference stream is high and relatively constant (large F value); and that DNA structural integrity of the sunfish from East Fork Poplar Creek improved during the study period to reach levels similar to those for Hinds Creek. In all probability, the large genotoxic response observed in sunfish from East Fork Poplar Creek during the years 1987 and 1988 (small F value) was related to the release of chemicals from the USDOE reservation. Diminution of this response in subsequent years may be due to the remedial activities that occurred on the USDOE reservation that attenuated the release of pollutants. Included in these activities were the capping of existing settling basins, the creation of a new settling basin, and the treatment of waste water before discharge. However the possibility that there has been an adaptive response over time by the resident population of sunfish to their environment can not be excluded (see discussion below on population genetics).

It is often difficult to relate effects observed in the field to the contaminants themselves or their source found in the environment because of the influence of noncontaminant-mediated factors. In such instances, laboratory studies may sometimes be important for establishing a chain of causality. For example, sunfish were exposed in the laboratory to sediment from East Fork Poplar Creek for 16 weeks to determine whether this was the major

source of genotoxicants for the native population of sunfish (14). Sediment-exposed sunfish showed a time-dependent increase in the level of strand breakage of their DNA. Also, other biologic responses of toxicologic relevance were measured and correlated with the genotoxic response (e.g., stress proteins and detoxication enzyme induction, metabolite in the bile, change in chromosomal proteins, etc.). Such information can be used not only to verify the source of environmental contamination (sediment in this case), but also to define cellular mechanisms that respond to genotoxic stress, which in turn may lead to a better understanding of the consequences of genotoxic exposure. During this laboratory investigation, several techniques for measuring strand breaks in DNA were compared. As a result, strand-break analyses in DNA from nonmammalian environmental species such as fish, birds, and amphibians are now being supplemented in our laboratory by agarose gel electrophoresis, an analytic technique that can provide quantitative data on both double-

and single-strand breaks present in the DNA molecule (15).

UVB-induced Photoproducts in DNA of Plants

In addition to its application to chemical contamination, genetic toxicology can also address concerns about possible adverse effects of enhanced ultraviolet-B (UVB) radiation (290 to 320nm) on the growth, reproduction, and survival of plants. Decreasing stratospheric ozone levels will result in an increase in net UVB radiation at the earth's surface. For example, a 10% decrease in stratospheric ozone could result in a 20% increase in UV penetration at 305 nm and a 250% increase at 290 nm (16). The large proportional increase in the shorter wavelength region (below 300 nm) of the UVB spectrum is of concern because of its ability to disrupt physiologic function and the likely induction of DNA damage in the form of pyrimidine dimers. Although UV radiation below 300 nm is extremely difficult to measure because it makes up only 1% of the UV that reaches the surface of the earth, this portion of the UV spectrum has been postulated to have had a major impact on the evolution of life on the planet (17).

A UVB exposure and monitoring system (18) was established at ORNL to deliver specific but adjustable levels of UVB radiation, to allow investigation of the effects of this type of radiation on plants and other biota in the environment. Preliminary results (19) using this exposure system over a 2-month period with two cultivars of soybean exposed to elevated UVB (32% above ambient) are summarized in Table 2. Changes in biomass and UV-absorbing compounds (secondary metabolites that attenuate ultraviolet light within plant tissue) were documented. One cultivar (Forrest) was found to be sensitive to elevated UVB as demonstrated by a decrease in biomass and UV-absorbing compounds while the resistant one (Essex) showed no change in biomass but an increase in UV-absorbing compounds. In

Table 2. Change in biological responses of soybean cultivars exposed to elevated UVB radiation.

Cultivar	Biomass	UVB-absorbing compounds, dimers	DNA damage, strand breaks
Forrest (14%)			Decreased
(25%)	Increased	Increase	Decreased
Essex (13%)	No change		Increased
	No change	Slight increase	

Plants were exposed for 2 months with the exposure and monitoring system (18) set to deliver 32% above ambient UVB radiation and to simulate daily and seasonal changes in solar irradiance with adjustment for cloud/canopy conditions.

addition, it was observed that total DNA damage (strand breaks and pyrimidine dimers) was 4.6 times greater in the sensitive cultivar vs the resistant one (19).

New Research Initiatives at ORNL

New research initiatives in genetic ecotoxicology are underway at ORNL to examine changes at the gene level that may be responsible for an organism's response to genotoxicants. These investigations are based on two important assumptions: that there may be a genetic basis for this response, and that techniques of molecular biology are available with the sensitivity and specificity to address questions about organism-toxicant interactions at the gene level. Two new initiatives are briefly discussed to illustrate the direction of our research.

Transgenic Fish

A transgenic fish (Japanese medaka, *Oryzias latipes*) has been produced containing the *lacZ* reporter gene through electroporation of medaka eggs at the four-cell stage of development. Currently, backcross matings to wild-type medaka have begun to detect integration of a single transgene and to establish inheritance in a Mendelian fashion. The transgenic fish will be used to determine the mutagenic potential of aquatic environments. For example, the *lacZ* can be retrieved from the transgenic fish after exposure and analyzed for change in mutational frequency. Also, the organism can be used to test for tissue susceptibility to genotoxic/mutagenic compounds or their metabolites and to detect specific DNA base changes caused by genotoxic agents.

Population Genetics

The effect of environmental contamination on population genetics of aquatic species is under investigation. This research is based on the hypothesis that there will be a selective advantage to variants in the population that are genetically predisposed to cope with toxicants. For example, we have been examining a series of retention ponds heav-

ily contaminated with radionuclides, but which have supported a resident population of mosquitofish (*Gambusia affinis*) for the past 20 years.

In a recent study (unpublished data) we found that there was an inverse correlation between DNA strand breakage and fecundity of fish from the contaminated ponds. This has implications for higher-order ecologic effects, as well as for contaminant-induced selection of resistant phenotypes. Current investigations have provided evidence that genetic diversity is increased in the population of fish occupying the radionuclide-contaminated sites relative to reference sites. These findings are supported both by allozyme analysis—through determination of average heterozygosity and percent polymorphisms—and by the RAPD (randomly amplified polymorphic DNA) technique—by determining average similarities of banding patterns between individuals within populations. In addition, it has been found that certain banding patterns are more prevalent in the contaminated sites than in the reference sites. Individuals that display these banding patterns at one of the contaminated sites have a higher fecundity and lower degree of strand breakage than individuals with the less common banding patterns. This type of pattern is also observed with allozyme analysis—heterozygotes, especially at the nucleoside phosphorylase locus, are more common in the contaminated sites. Within the contaminated sites, heterozygotes have a higher fecundity and lower degree of strand breakage than do homozygotes. Long-term laboratory exposures where environmental variables can be more rigidly controlled are underway in an effort to establish relationships among genotype, DNA strand breakage, and fecundity.

Discussion

This report summarizes several past attempts at ORNL to detect genotoxic insult in environmental species exposed to pollution and outlines current investigations to predict or define the potential consequences at higher levels of organization (e.g., population). The former studies

examined DNA for structural modifications indicative of damage caused by a genotoxic agent (adduct, strand breakage, and photoproduct). The data were then applied to a particular environmental problem. For example, with the beluga whale, the data helped stimulate the debate on how to manage a threatened species in a polluted environment (7). At the USDOE reservation in Oak Ridge, Tennessee, the data have been used to define hazardous environments (turtle studies) or to monitor the effectiveness of activities associated with remediation (sunfish studies).

Even though genetic toxicologic investigations are important for the documentation of exposure, they often fail to provide the information necessary to establish why the insult occurred or the outcome. Ancillary data can help ameliorate this situation by defining other cellular mechanisms associated with or linked to the genotoxic response. For example, the difference noted in the amount of UVB-type damage to the DNA of two soybean cultivars could be explained to some extent by the increase in UV-absorbing compounds in one cultivar but not the other (19). Nevertheless, none of these observations explains the effect of UVB exposure on biomass in these plants.

It is obvious that new approaches are needed that address questions of ecologic significance. Our studies with a population of *G. affinis* introduced into a radionuclide contaminated pond show that acclimation and adaptation to environmental stress occurred. These processes have a genetic basis; therefore, understanding change at the genetic level should help identify the more complex changes at higher levels. Application of experimental tools currently in use in molecular biology and other related disciplines should help in our understanding of key biologic mechanisms that regulate and limit the response of organisms to stresses in their environment. This is a fruitful area for genetic ecotoxicologic research, because it offers an opportunity to rapidly advance our knowledge and understanding of the effect of environmental pollution (20).

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