

Linking Genotoxic Responses and Reproductive Success in Ecotoxicology

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The potential of genotoxicity biomarkers as predictors of detrimental environmental effects, such as altered reproductive success of wild organisms, must be rigorously determined. Recent research to evaluate relationships between genotoxic responses and indicators of reproductive success in model animals is described from an ecotoxicological perspective. Genotoxicity can be correlated with reproductive effects such as gamete loss due to cell death; embryonic mortality; and heritable mutations in a range of model animals including polychaete worms, nematodes, sea urchins, amphibians, and fish. In preliminary studies, the polychaete worm, *Neanthes arenaceodentata*, and the nematode, *Caenorhabditis elegans*, have also shown the potential for cumulative DNA damage in gametes. If DNA repair capacity is limited in gametes, then selected life history traits such as long and synchronous periods of gametogenesis may confer vulnerability to genotoxic substances in chronic exposures. Recommendations for future research include strategic development of animal models that can be used to elucidate multiple mechanisms of effect (multiend point) at varying levels of biological organization (multilevel). — Environ Health Perspect 102(Suppl 12):9–12 (1994)

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Introduction

Genetic ecotoxicologists are interested in five detrimental outcomes of exposure to genotoxic substances. These include increased frequencies of: gamete loss due to cell death, embryo mortality (lethal mutations), abnormal development, cancer, and heritable mutations which may cause either increased or decreased genetic diversity. In ecotoxicology, effects on individuals are generally not as significant as they are in human toxicology. Consequently, a primary goal of ecotoxicology is to relate effects manifested in individuals to changes in population size or structure. Altered fertility, development, and embryonic survival are environmentally significant, because they can reduce reproductive success and thus alter population size or structure. Nevertheless, little has been done to explore linkages between genotoxic responses and resultant reproductive and developmental effects.

Research to understand the relationships between genotoxic responses and measures of reproductive success has its roots in an

extensive literature on nonmammalian animal models in radiobiology and chemical carcinogenesis. For example, Schroeder (1) reviewed much of the early literature on radiation-induced mutations in fish. Others (2) have reviewed research on germ-cell mutations in animals such as *Drosophila melanogaster*. However, this work was not directed toward ecotoxicological problems. Thus, many of the successes in aquatic radiobiology have never been adapted for evaluation of chemical effects, and model animals such as *Drosophila* cannot be used for exposures in media such as soil, sediment, and water. It is vital that we build upon findings of previous studies as well as continue to develop and improve effective techniques for addressing ecotoxicological problems.

With the development of new animal models in genetic ecotoxicology, new relationships between genotoxic responses and measures of reproductive success are beginning to emerge. These recent advancements set the stage for rapid progress in this field in the years to come. We present an overview, from an ecotoxicological perspective, of recent research relating genotoxic responses and measures of reproductive success in key animal models. In addition, selected principles that could accelerate progress in this area of research are discussed.

The Polychaete Worm *Neanthes arenaceodentata*

Using the polychaete, *Neanthes arenaceodentata*, correlations between cytogenetic effects and decreased fertility have been observed. These studies have also provided

evidence that certain life history traits may confer vulnerability to genotoxic substances (3). Harrison et al. (4) initiated research into the cytogenetic effects of ionizing radiation. They determined that chromosomal aberrations and sister chromatid exchanges (SCE) were induced in larvae of *N. arenaceodentata* at 2.0 Gy and 0.6 Gy, respectively. In a subsequent study, *N. arenaceodentata* were exposed to several doses of gamma radiation, and the doses at which chromosomal aberrations were induced (in mixed tissues of juveniles) were compared to the doses at which broodsize alterations and lethality occurred in both irradiated adults and irradiated juveniles (5). At 2.0 Gy, significant increases in chromosomal aberrations were observed again. Significant decreases in broodsize were observed at 4 Gy in worms irradiated as adults and at 8.4 Gy in worms irradiated as juveniles; in contrast, lethality occurred at much higher doses of 500 Gy. It was hypothesized that chromosomal aberrations caused cell death in gametes and subsequent decreases in broodsize. It would be expected that cytogenetic effects could be detected at lower levels than broodsize decreases, because some cytogenetic changes, such as small chromatid deletions, would not always be lethal to cells.

Refined estimates of the acute doses of ionizing radiation that caused both decreased fertility and increased embryonic mortality were developed later. In this study (6), in which adult *N. arenaceodentata* were irradiated, broodsize decreases were only observed between 5 and 10 Gy, but embryonic mortality was observed at much lower

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doses of 0.5 Gy. Significantly, the doses at which embryonic mortality was induced were similar to those at which SCE were induced in the first study. Because SCE have been correlated with mutagenesis (7), it is possible that these are predictive of the levels at which lethal mutations are induced in embryos. These data suggest that embryonic mortality might have been attributable, at least in part, to lethal mutations in embryos and that lethal mutations in embryos may be more sensitive indicators of reproductive impairment than are alterations in fertility. For *N. arenaceodentata*, Anderson and Harrison (3) have reviewed the mechanistic linkages between genotoxic responses and measures of reproductive success as well as the factors such as cell cycling and gametogenic stage that may modify effect-level estimates.

Chronic-exposure experiments were also conducted (8). Comparison of the embryonic survivorship data obtained from acute and chronic exposures revealed that DNA repair may not be active in gametes of this species. For total doses of 10 Gy or less, rates of embryonic mortality observed in chronic exposures were the same as those observed following acute exposures of the same total dose. These data indicate that DNA damage may be cumulative in oocytes of *N. arenaceodentata*, which leads to the hypothesis that organisms with long synchronous periods of gametogenesis may be more vulnerable to cumulative effects of chronic exposure to genotoxic substances, depending on the repair capacities of oocytes.

The Nematode *Caenorhabditis elegans*

The nematode *Caenorhabditis elegans* is an excellent animal model for evaluating relationships between genotoxicity and reproductive success. Virtues of this species include: availability of detailed information regarding genetics, reproduction and development (9), a short generation time of 4 to 5 days, hermaphroditic reproduction which negates the need to conduct matings, and availability of procedures for long-term culture and mutagenesis research (10). In addition, cytogenetic methods are available for embryonic nuclei (11).

Recent experiments with *C. elegans* highlight the importance of considering a diversity of genotoxic responses and reproductive outcomes in assessing the effects of mutagenic substances. For example, experiments in which young adult worms were exposed to ethyl methane sulfonate (EMS) at a developmental stage in

which both mature sperm and primary oocytes had been formed have shown that significant increases in mutation frequencies were observed at doses as low as 0.5 mM EMS. Yet, a significant decrease in the number of viable progeny occurred only at doses in excess of 45 mM (12). Because not all mutations will cause embryonic death or decreased fertility, the low-level effects of some substances may be more likely to be related to heritable mutations than to reproductive success. In contrast, experiments with ultraviolet-B (UV-B) have shown that both broodsize may decrease and the frequency of sterile worms may increase at doses that do not cause detectable mutations in some mutagenesis assays. Specifically, ongoing research in which *C. elegans* are continuously exposed to UV-B (using the irradiation techniques of Karentz et al. (13)) over 12 generations, has shown that broodsize decreases by 50% in the first generation and remains stable at the 50% level throughout all subsequent generations. In contrast, the frequency of sterile worms increased in every generation after the first six generations of exposure. Using an assay that targets a 350-gene region (10), we quantified induced mutations in nematodes removed from the multigeneration exposure experiment after 12 generations of exposure. No increase in mutation frequencies over controls was observed. The mechanism of generating sterile worms is unknown and although a genetic effect is strongly suggested, this was not supported by the results of this particular mutagenesis assay.

Similar to the polychaete studies, putative cumulative dose effects were observed in the nematode. When *C. elegans* (with mature sperm and primary oocytes) were exposed to 10 mM EMS for 1, 4, 8, and 24 hr, a dose-dependent increase in mutation frequencies was observed (Table 1). A rigorous test of the extent to which long-term, low-level exposures to mutagens may be accumulated in gametes would require direct assessments of the kinetics of absorbed dose and of DNA repair in gametes. In the mouse, it is believed that DNA repair is not active in

postmeiotic cells (14), and thus precedent exists for low DNA repair capacities in selected gametogenic stages of some organisms. Further experimental assessments would greatly increase our understanding of the factors that confer vulnerability to genotoxic substances as well as of the potential for low-level exposures to cause significant environmental harm. Because controversy often surrounds prediction of low-dose effects in environmental risk assessment, further mechanistic research could have important implications in environmental management.

The Sea Urchin *Strongylocentrotus purpuratus*

The sea urchin, *Strongylocentrotus purpuratus*, is an ideal species for evaluating relationships between cytogenetic responses in embryos and abnormal development. Hose (15) adapted an anaphase aberration technique to aquatic embryos which has proved a practical assay for assessing genotoxicity. In addition, development and fertilization responses in this species have been studied widely and used in bioassays conducted for environmental regulation.

Recently, the sensitivity of the anaphase aberration assay was compared to that of abnormal development in embryonic *S. purpuratus* (16). These were also compared to the sensitivity of the fertilization end point, which is most commonly used in environmental management (Table 2). For phenol, anaphase aberrations were induced at doses that did not significantly inhibit development. In contrast, pentachlorophenol exposure induced anaphase aberrations only at doses higher than those that elicited abnormal development. Benzidine exposure resulted in significant increases in anaphase aberrations and abnormal development at the lowest dose tested. For these three chemicals, fertilization was never the most sensitive end point. These data highlight the need for both development and genotoxicity assays in ecological risk assessment. The development test clearly predicts toxicity associated with mechanisms which may include genetic effects. On the other hand,

Table 1. Mutagenesis in the nematode *Caenorhabditis elegans* following exposure to 10 mM EMS at four time points.^a

Exposure time, hr	No. F ₁	No. mutants	Mutant frequency, × 10 ⁻²
1	258	5	1.9 ± 0.9
2	251	9	3.6 ± 1.2
8	286	23	8.1 ± 1.7
24	195	34	17.0 ± 3.0

^aThe assay was conducted according to the methods of Rosenbluth et al. (10) using JPI0 nematodes. The laboratory control value for mutation frequencies in this assay is $0.2 \pm 0.2 \times 10^{-2}$.

Table 2. Lowest observed effect concentrations (LOEC) for fertilization, development, and anaphase aberrations in embryos of the sea urchin, *Strongylocentrotus purpuratus*, exposed to three mutagenic chemicals (concentrations in mg/l) after Anderson et al. (15).

Chemical	Fertilization	Development	Anaphase aberrations
Phenol	50.0	>20.0	2.5
Pentachlorophenol	0.5	<0.1	<0.1
Benzidine	10.0	<1.0	10.0

the genotoxicity assay can be more sensitive and may be predictive of latent effects that will not be realized until later stages of development. To our knowledge, stage-specific expression of genotoxic effects has never been evaluated in aquatic organisms.

The sea urchin has also been used to evaluate the developmental and genotoxic effects of increasing UV-B as a consequence of Antarctic ozone depletion (17). Antarctic sea urchins, *Stereochinus neumayeri*, were exposed to UV-B in laboratory settings and *in situ* at Palmer Station Antarctica. Development, cellular abnormalities, and cytogenetic effects were assessed. Data from this study have shown that under ambient ozone column conditions (e.g., not depleted), UV-induced damage can be significant in surface exposures and that attenuation of effects with depth can be monitored. These data highlight the fact that assays to evaluate multilevel effects can also be deployed for *in situ* experimentation. Expanded development of multilevel, *in situ* experimentation will add realism to our assessments of genotoxic and reproductive effects as current relative relationships may easily be modified by environmental factors such as ambient ultraviolet light, temperature, and oxygen tension.

Vertebrate Models

Linkages between genotoxicity and reproductive success have been evaluated in a variety of fish species. Longwell has pioneered this area of research with evaluations of mitotic abnormality and embryo mortality in Atlantic mackerel, *Scomber scombrus* (18). A positive correlation between anaphase aberration frequencies and developmental abnormalities was also obtained using trout embryos (19). Subsequently, studies of larval herring (20) have shown that anaphase aberrations are positively correlated with developmental abnormalities but also may be related to success of individual year classes. The induction of dominant lethal mutations by radiation and chemicals has also been evaluated in a variety of fish species. The most extensive research has been conducted with the Japanese medaka, *Oryzias latipes*

(21,22). We are now expanding on this research with studies of female germ cell mutagenesis. Eventually, we hope to use the medaka for studies elucidating mechanisms of oocyte loss and embryo mortality.

Recently, genotoxic effects on amphibians are being considered in our laboratory from an ecological perspective. Although other investigators have evaluated genotoxic effects in amphibia exposed to mutagenic chemicals (23), none have determined whether genotoxic responses were predictive of detrimental reproductive effects. Studies are underway to determine whether there are correlations between frequencies of micronuclei in circulating erythrocytes, DNA adducts in liver, wet weight at metamorphosis, and time to metamorphosis of *Xenopus laevis* tadpoles following exposure to benzo[a]pyrene. Time to metamorphosis is related to fitness due to the necessity for larvae to attain metamorphosis before larval habitats become dry (24,25). Size at metamorphosis can be associated positively with reproductive success (26). Traits that relate to the fitness of an individual as it concerns reproductive success can be numerous, and the complex interrelationships between these traits and genotoxic responses remain almost totally unexplored.

Summary and Recommendations

This article has raised three key points that will have bearing on the development of research into linkages between genotoxic responses and reproductive effects in ecotoxicology. These are: *a*) Mutations are associated with gamete loss, abnormal development, embryonic mortality, or heritable mutations in a variety of animal models. These effects can be related to reproductive success directly or indirectly via ecological correlates. *In situ* monitoring of genotoxic responses in animals will be accelerated as the significance of genetic biomarkers is established and related to detrimental reproductive effects. *b*) Life history traits conferring vulnerability to genotoxic substances should be elucidated in mechanistic studies which will aid in rational selection of model animals. *c*) A diver-

sity of biological end points and experimental designs must be carefully considered in studies relating genotoxic and reproductive responses. For example, mutations, sterility, embryonic mortality, cell death, and epigenetic alterations may arise in one system or exposure but not in others. Assays and monitoring strategies could be designed in a more parsimonious manner if mechanistic understandings of the most responsive end points for specific exposure conditions were developed and such end points proved to be consistently responsive.

These key points lead to the following specific recommendations for strategic development of animal models for genetic ecotoxicology research. Most research cited herein into development of assays using model animals has taken into account factors such as ease of culture, knowledge and manipulability of reproductive cycle, availability of genotoxicity test techniques, and practical applications to diverse media. In some instances, more sophisticated concerns such as vulnerable life history strategies (3), amenability to multilevel or multigeneration experimentation [research in progress (5,12,16,17)], and feasibility of assessing multiple genotoxic and reproductive end points have been considered. However, additional considerations must be addressed. For example, DNA repair capacity, chromatin configuration, and susceptibility of gametogenic stages may all have a bearing on the potential sensitivity of an animal model (27). In addition, the appropriateness of an animal model as a candidate for the implementation of modern molecular techniques should be considered. It is recommended that further development of animal models maximize the potential for multiend point and multi-level investigations for acute (high dose, short exposure) and chronic (low dose, long exposure) exposures, the vulnerability of life history traits be examined, direct assessments of damage to germ cells be encouraged, and selected development of modern molecular genetic techniques be considered an essential priority.

As we move toward more strategic development of the field of genetic ecotoxicology, we accelerate our progress toward our ultimate goals. These are to assess genotoxic damages *in situ* and understand their significance, to provide realistic predictions of the biological effects of genotoxic substances before they are discharged, and to understand how toxicant-induced changes in genomes and gene pools might affect the long-term survival of populations.

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