Large-scale Genotoxicity Assessments in the Marine Environment

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There are a number of techniques for detecting genotoxicity in the marine environment, and many are applicable to large-scale field assessments. Certain tests can be used to evaluate responses in target organisms in situ while others utilize surrogate organisms exposed to field samples in short-term laboratory bioassays. Genotoxicity endpoints appear distinct from traditional toxicity endpoints, but some have chemical or ecotoxicologic correlates. One versatile end point, the frequency of anaphase aberrations, has been used in several large marine assessments to evaluate genotoxicity in the New York Bight, in sediment from San Francisco Bay, and following the Exxon Valdez oil spill. — Environ Health Perspect 102(Suppl 12): 29-32 (1994)

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Introduction

With the advent of genotoxicity techniques suitable for evaluating effects on marine organisms, several large-scale field assessments have been performed (1-6). These and many other smaller studies (7-10) have demonstrated associations between genetic damage and marine pollution, and some have attempted to link their findings to reproductive success or adverse population effects (3,5). The ecotoxicologic consequences of genetic damage must be defined to demonstrate that the measurement of genotoxicity is environmentally useful.

In the marine environment, we are presented with unique challenges in the determination of environmental injury compared to scientists studying terrestrial or freshwater systems. It is obvious that not being able to visually track the fate of discharges and target organisms greatly complicates injury determination. Most large marine animals are mobile and are capable of avoiding noxious conditions, and this mobility makes estimation of exposure extremely difficult. Marine organisms also have reproductive strategies which frustrate population biologists. For instance, many commercially important marine animals annually produce thousands to millions of eggs, of which only a fraction must become reproducing adults to maintain the population. Reproductive success can be quantified, but it usually has no relation to future population size (11-13). To accurately determine population injury, assessments must continue until the fish has grown to a size at which it can be systematically monitored, usually 3 to 5 years. Few funding agencies are willing to commit to such long-term endeavors, so population-level impacts are rarely documented in the marine environment.

One approach to injury determination is to assume that damage to somatic cells is a predictor of germ cell damage. This assumption has not been validated but appears logical based on mammalian research (14,15). Embryonic and early larval life stages are critical in terms of environmental injury since the embryo undergoes differentiation, including germ cell formation (16). The preceding assumption appears more feasible in the case of marine embryos and larvae because their small size facilitates distribution of the mutagen throughout the body. In adults, contaminants are differentially partitioned into the gonads, and actual exposure to the germ cells may be further modified by metabolism of the contaminant. It is currently impossible to determine the ecologic consequences of all but the most obvious germ cell damage (17). The ability to evaluate a F1 generation is limited to a relatively few species which can be artificially spawned. Prediction of reproductive impairment from measurement of germ cell damage is further complicated for marine organisms, most of which do not have a determinate number of oocytes.

A second method is to utilize genotoxicity bioassay organisms as surrogates for target species. The assumption that the sensitivity of bioassay organisms brackets those of the target species is already used for traditional toxicity bioassays (18). A variety of in vitro tests are thus available for marine animals (2,4,7-9,19); however, no tests using marine plants currently exist. One advantage to the bioassay paradigm is that certain tests such as the Mutatox test (20) could be useful for evaluating large numbers of samples and for samples with small volumes such as pore water. This type of assay is thus amenable to large-scale field applications but requires further validation. The sensitivities of short-term tests like Mutatox and the Ames test in comparison to those of target organisms need to be defined since metabolic activation patterns are phylogenetically diverse and not easily predicted (21). One disadvantage of in vitro genotoxicity assays is that they rarely simulate real-life exposures. Early life stages of marine organisms are usually not subjected to continuous exposure since they are carried by ocean currents. Static exposure conditions thus are rarely indicative of real-life exposures, and the effects of pulsed exposures vary depending on the developmental stage of the organism (16).

Therefore, in situ evaluations of target organisms would seem to provide the most realistic information on genetic damage. Difficulties with this approach are exemplified again by marine fishes, the critical life stages of which are positively buoyant (planktonic), tiny eggs which hatch into equally tiny larvae. The logistics of collecting and identifying eggs and larvae in similar developmental stages from disparate locations frequently necessitate chemical
preservation is also the case for the evaluation of archived organisms to yield historical baseline data. However, preservation with formaldehyde is incompatible with most DNA integrity measurement techniques (7–9) and those capable of documenting exposure to specific genotoxins such as DNA adduct formation (22). One cyto genetic technique, the measurement of mitotic aberrations in anaphase-telephase cells, is compatible with these types of samples, is known to be sensitive to environmental mutagens, but is nonspecific with regard to exposure (21,23). This review will focus on three large-scale field studies employing the anaphase aberration test. Each utilizes a multidisciplinary or multiorganism approach to the detection of genotoxic substances in the marine environment. Results from the Exxon Valdez oil spill herring study have been linked with population data to determine the long-term consequences of genetic damage (24).

**Case Study 1: New York Bight Mackerel Study**

In the late 1970s, Longwell et al. (1,25) surveyed embryos of Atlantic mackerel (Scomber scombrus) throughout the New York Bight for mitotic (including anaphase) aberrations, mortality, and gross malformations (Table 1). Higher mortalities were found along the coastal regions near the New York Bight apex, off New Jersey, and near a dredge spoil dump site. The frequencies of mitotic aberrations, mortality, and malformations in early (epiboly) embryos were intercorrelated; their spacial distributions were similar. Correlations were performed between the biologic end points, environmental variables, and chemical contaminants in surface water and plankton (Table 2). Throughout the entire period of mackerel embryogenesis, aromatic hydrocarbons were associated with mortality and mitotic measurements. Heavy metals were associated with malformations of embryos midway through embryogenesis, and chlorinated hydrocarbons with effects in late embryos. Of the environmental measurements, temperature was inversely correlated with all end points, stressing the need for considering natural variability in large-scale field studies. Salinity was negatively correlated with effects in the early developmental stages; however, effluents are less saline than seawater.

A second technique, the blood micronucleus test which measures chromosome/chromatid breakage in juvenile or adult fish, complemented the embryolarval study. Frequencies of micronucleated cells were highest in fish from the apex of the New York Bight and comparatively higher along the coastal mid-Atlantic than from other major Atlantic water masses (6). Although the use of the piscine micronucleus test has been controversial because of low discriminatory power (defined as the widest range of response and the lowest analytical variability independent of the use of controls), poor replication and the possibility of artifact due to intraerythrocytic viruses (26), it remains widely used in mammalian toxicology. In addition, little information is available regarding the regulation of erythropoiesis in fish by such basic elements as diet, disease, and water temperature.

Longwell et al. (1) speculate that the considerable embryo mortality they observed would lead to adaptation of some fish populations to contamination. The pressure to adapt to pollution would compete with genetic selection from climate change; disease; and, in economically important species, overfishing. As historic breeding grounds become lost to pollution, there will be increased pressure while adapting to the environmental dynamics of less optimal spawning areas. They stress that the ecotoxicologic consequences of environmental contamination, habitat destruction, and anthropogenic actions are cumulative.

**Case Study 2: San Francisco Bay Sediment Toxicity Study**

As part of a comprehensive *in vitro* evaluation of sediment toxicity in San Francisco Bay, California, genotoxicity end points were evaluated using two traditional marine bioassay organisms, the purple sea urchin (*Strongylocentrotus purpuratus*) and blue mussel (*Mytilus edulis*) (2,27).

Embryos were incubated in sediment extracts and anaphase aberrations measured. All 15 stations showed significantly elevated anaphase aberrations rates in mussel embryos, with 3- to 12-fold increases above sediment control values. Sediments from three sites, including the northern open water San Pablo Bay station, were among the least toxic. The majority of genotoxic sites were located in areas with low circulation: Inner Richmond Harbor, Oakland Inner Harbor, northern part of South Bay, and Redwood Creek. These results were not associated with the traditional mussel bioassay end points. Elevated embryonic abnormalities were observed at only three of the sites, and embryo survival was not affected at any site.

Using sea urchin embryos, anaphase aberration rates were higher at all of the stations except San Pablo Bay (2,27). Highly significant elevations in anaphase aberration rates were observed with sediment from Oakland Inner Harbor, northern South Bay, off Islais Creek, and Redwood Creek. Although only five samples were analyzed for chemical contaminants, correlation coefficients (r values) of ≥0.7 were obtained between the anaphase aberration rate and bay-region polycyclic aromatic hydrocarbons, polychlorinated biphenyls, DDE, and several heavy metals (Table 2). The discriminatory power was not high but was within the range of standardly used toxicity end points (27). Results were consistent with the mussel genotoxicity test and were more sensitive than traditional toxicity end points used in sediment quality management. Although developmental and genotoxic responses of sea urchins are associated in some instances, developmental toxicity can occur in the absence of genetic damage (28). It therefore appears that the genotoxicity end point is distinct from the traditional toxicity end points.

**Case Study 3: Exxon Valdez Oil Spill Herring Study**

On March 24, 1989, the tanker Exxon Valdez spilled at least 11 million gallons of crude oil into Prince William Sound, Alaska. The current rapidly dispersed the oil toward the western shores of the Sound. Pacific herring (*Clupea pallasi*) migrated into spawning areas and deposited their eggs by April. The embryos hatched in early May, and the larvae were evaluated for gross malformations and genetic damage (Table 1). Eggs collected from unspooled beaches at Fairmont Bay produced larvae with low anaphase aberration rates (5,29).

| Study          | End points                  | Correlates                          |
|----------------|-----------------------------|-------------------------------------|
| Longwell et al. (1) | Mitotic abnormalities | Embryo mortality |
| Longwell and Hughes (25) |                    | Gross malformations               |
| Norcross et al. (5) | Anaphase aberrations      | Adult micronucleus formation in erythrocytes |
| Hose et al. (29)  |                            | Embryo mortality                  |
|                |                            | Gross malformations               |
|                |                            | Decreased adult survival to recruitment |
Aberration rates were significantly elevated at five oiled beaches and were highly correlated with the Exxon Valdez oil polycyclic aromatic hydrocarbon concentration as well as with total aromatic hydrocarbon and phytane concentrations (Table 2). Genetic damage in free-swimming larvae was less than in newly hatched larvae; but it was elevated near oiled spawning beaches with greatest frequencies of anaphase aberrations near the heavily oiled sites. Aberration rates were lower in two- to four-week old larvae but remained elevated at sites within the oil trajectory (5). Genetic effects and gross malformations attributable to residual oil were undetectable in 1990 and 1991 (29).

Genetic and ecotoxicologic effects were linked in the exposed 1989 year class. Reduced survival from egg to hatching was observed at oiled areas. The effect could not be solely attributed to oil exposure since there is no independent information on natural egg loss rates at these sites (30). Larval malformation rates were elevated at oiled sites (29), and larval growth rates were extremely low throughout Prince William Sound in spring 1989 (5).

Although the 1989 year class was forecast to be weak, it is one of the smallest recorded in 20 years (24). In 1993 to 1994, the spawning population was primarily composed of 1988 year class fish, some of which might have been exposed to oil as 1 year olds feeding in contaminated nearshore areas. Their abundance was only 25 to 50% of that expected, and mortality rates doubled, apparently due to a viral epizootic (24).

Germ cell (heritable) damage has not been thoroughly investigated; however, a preliminary study of the 1988 year class suggests possible reproductive impairment of individuals from previously oiled areas (31). Although the baseline of anaphase aberrations in fish from unoiled areas doubled to 20% by 1991, natural factors (such as cool temperatures with many storm events, resuspension of sediment, higher egg densities, and mortality) during embryogenesis appear responsible (24). This study is the first to link genotoxicity to population level responses.

Conclusions

Aquatic genotoxicity measurements are envisioned to be useful indicators of long-term ecotoxicologic effects. Techniques are available to monitor genetic damage in individuals and possibly in populations of marine organisms; however, there is a need for techniques which are robust statistically and with respect to the types of media and organisms which can be tested. Notably lacking is interpretable measurement of germline damage in aquatic organisms. In addition, we are still missing much of the information needed to link short- and long-term effects. Future research should also focus on the relationship of in vitro bioassays to population and ecosystem changes as well as the role of contamination in the overall adaptive capacity of organisms. Once these answers are available, guidelines can be established for the prevention or remediation of ecotoxicologic damage.

### Table 2. Chemical correlates of genetic toxicity tests.

| Study                  | End points                      | Correlates                                      |
|------------------------|---------------------------------|-------------------------------------------------|
| Longwell et al. (1)    | Mitotic abnormalities           | Aromatic hydrocarbons                           |
| Longwell and Hughes (25)|                                 | Chlorinated hydrocarbons                        |
| Long et al. (2)        | Anaphase aberrations            | Bay region polycyclic aromatic hydrocarbons DDE|
| Long and Markel (27)   |                                 | Polychlorinated biphenyls                       |
| Hose et al. (29)       | Anaphase aberrations            | Exxon Valdez Oil-polycyclic aromatic hydrocarbons|
|                        |                                 | Total aromatic hydrocarbons                     |
|                        |                                 | Phytane                                         |

### REFERENCES

1. Longwell AC, Chang S, Hebert A, Hughes JB, Perry D. Pollution and developmental abnormalities of Atlantic fishes. Environ Biol Fish 35:1–21 (1992).
2. Long ER, Buchman MF, Bay SM, Breteler RJ, Carr RS, Chapman PM, Hose JE, Lissner AL, Scott J, Wolfe DA. Comparative evaluation of five toxicity tests with sediments from San Francisco Bay and Tomales Bay, California. Environ Toxicol Chem 9:1193–1214 (1990).
3. Stein JE, Collier TK, Reichert WL, Casillas E, Hom T, Varanasi U. Bioindicators of contaminant exposure and sublethal effects: studies with benthic fish in Puget Sound, Washington. Environ Toxicol Chem 11:701–714 (1992).
4. Stiles S, Choromanski J, Nelson D, Miller J, Greig R, Sennefelder G. Early reproductive success of the hard clam (Mercenaria mercenaria) from five sites in Long Island Sound. Estuaries 14:332–342 (1991).
5. Norcross BL, Frandsen M, Hose JE, Biggs E. Larval herring distribution, abundance and sublethal assessment in Prince William Sound, Alaska during 1989 following the Exxon Valdez oil spill. Can J Fish Aquat Sci (in press).
6. Hughes JB, Hebert AT. Erythrocyte micronuclei in winter flounder (Pseudopleuronectes americanus): results of field surveys during 1980–1988 from Virginia to Nova Scotia and in Long Island Sound. Arch Environ Contam Toxicol 20:474–479 (1991).
7. Nacci D, Jackim E. Using the DNA alkaline unwinding assay to detect DNA damage in laboratory and environmentally exposed cells and tissues. Mar Environ Res 28:333–337 (1989).
8. Shugart LR. Quantitation of chemically induced damage to DNA of aquatic organisms by the alkaline unwinding assay. Aquat Toxicol 13:43–52.
9. DiGiulio RT, Habig C, Gallagher EP. Effects of Black Rock Harbor sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. Aquat Toxicol 26:1–22 (1993).
10. Kurelec B. The genotoxic disease syndrome. Mar Environ Res 35:341–348 (1993).
11. Powers DA, Chapman R, Chen TT. A molecular approach to recruitment problems: genetics and physiology. In: Toward a Theory on Biological-Physical Interactions in the World Ocean (Rothschild BJ, ed). Boston:Kluwer Academic Publishers, 1988:411–440.
12. Kawasaki T, Tanaka S, Toba Y, Taniguchi A. Long-term Variability of Pelagic Fish Populations and Their Environments. Oxford:Pergamon Press, 1991.
13. Jones R, Henderson EW. Simulation studies of fish larval survival. In: Toward a Theory on Biological-Physical Interactions in the World Ocean (Rothschild BJ, ed). Boston:Kluwer Academic Publishers, 1988:343–372.
14. Crisp TM. Organization of the ovarian follicle and events in its biology: oogenesis, ovulation or arresta. Mutat Res 296:89–106 (1992).
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15. Russell LB, Russell WL. Frequency and nature of specific-locus mutations induced in female mice by radiation and chemicals: a review. Mutat Res 296:107–127 (1992).
16. von Westernhagen H. Sublethal effects of pollutants on fish eggs and larvae. In: Fish Physiology, the Physiology of Developing Fish, Vol 11A. Eggs and Larvae (Hoar WS, Randall DJ, eds). San Diego: Academic Press, 1988; 253–346.
17. Johnson L, Casillas E, Sol S, Collier T, Stein J, Varanani U. Contaminant effects on reproductive success in selected benthic fish. Mar Environ Res 35:165–170 (1993).
18. Weber CI, Horning WB II, Klemm DJ, Neiheisel TW, Lewis PA, Robinson EL, Menkedick J, Kessler F. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA-600/4-87/028. Springfield, VA: National Technical Information Service.
19. Kocan RM, Landolt ML, Bond JA, Bendor EP. In vitro effect of some mutagens/carcinogens on cultured fish cells. Arch Environ Contam Toxicol 10:663–671 (1981).
20. Johnson BT. An evaluation of a genotoxicity assay with liver S9 for activation and luminescent bacteria for detection. Environ Toxicol Chem 11:473–480 (1992).
21. Anderson SL, Hose JE, Knezovich JP. Genotoxic and developmental effects in sea urchins are sensitive indicators of effects of genotoxic chemicals. Environ Toxicol Chem 13:1033–1041 (1994).
22. Ray S, Dunn BP, Payne JF, Fancey L, Helbig R, Beland P. Aromatic DNA-carcinogen adducts in beluga whales (Delphinapterus leucas) from the Canadian Arctic and the Gulf of St. Lawrence. Mar Poll Bull 22:392–396 (1991).
23. Kocan RM, Landolt ML, Sabo KM. Anaphase aberrations: a measure of genotoxicity in mutagen-treated fish cells. Environ Mutagen 4:181–189 (1982).
24. Brown ED, Baker TT, Funk F, Hose JE, Kocan RM, Marty GD, McGurk MD, Norcross BL, Short J. The Exxon Valdez oil spill and Pacific herring in Prince William Sound: a summary of injury from 1989-1994. In: Exxon Valdez Oil Spill Symposium (American Fisheries Society, Wildlife Society, Exxon Valdez Oil Spill Trustee Council, eds). Am Fish Soc Symp Ser (in press).
25. Longwell AC, Hughes JB. Cytologic, cytogenetic, and developmental state of Atlantic mackerel eggs from sea surface waters of the New York Bight, and prospects for biological effects monitoring with ichthyoplankton. Rapp P-v Reun Cons int Explor Mer 179:275–291 (1980).
26. Carrasco KR, Meyers MS. An assessment of the piscine micronucleus test as an in situ biological indicator of chemical contamination. Can J Fish Aquat Sci 47:2123–2136 (1990).
27. Long ER, Markel R. An evaluation of the extent and magnitude of biological effects associated with chemical contaminants in San Francisco Bay, California. NOAA Technical Memorandum NOS ORCA 64. Seattle: National Oceanic and Atmospheric Administration, 1992.
28. Bay S, Burgess R, Nacci D. Status and applications of echinoid (Phylum Echinodermata) toxicity test methods. In: Environmental Toxicology and Risk Assessment, ASTM STP 1179 (Landis WG, Hughes JS, Lewis MA, eds). Philadelphia: American Society for Testing and Materials, 1993; 281–302.
29. Hose JE, McGurk MD, Marty GD, Biggs ED, Baker TT. Sublethal effects of the Exxon Valdez oil spill on herring embryos and larvae: morphologic, cytogenetic, and histopathological assessments, 1989–1991. Can J Fish Aquat Sci (in press).
30. McGurk MD, Biggs ED. Egg-larval mortality of Pacific herring in Prince William Sound, Alaska, after the Exxon Valdez oil spill. Can J Fish Aquat Sci (in press).
31. Kocan RM, Marty GD, Baker T, Biggs E. Reproductive success in individual Prince William Sound herring three years after the Exxon Valdez oil spill. Can J Fish Aquat Sci (in press).