# GENOTOXICITY ASSESSMENT IN THE MARINE ENVIRONMENTS

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In addressing the issue of genotoxicity in marine biota, it is important at the outset to clarify the context in which research is undertaken in this area. Unlike medically oriented genotoxicity studies, where the key concern is the individual, ecologically oriented studies deal with the effects of pollutants on the genetic make up of natural population of organisms. Ecotoxicologists are interested in six detrimental outcomes of exposure to genotoxicants. ie. gamete loss due to cell death, embryo mortality (lethal mutation), abnormal development, neoplasia, heritable mutations which may cause increase or decrease in genetic diversity and changes in gene expression that effect Darwinian fitness. Pollutants may exert selective pressures which are reflected in changes in the genotypic constitution of populations. This is especially well illustrated by the evolution of genetically resistant populations at chronically polluted sites. Pollutant induced selection of resistant genotypes has been demonstrated for fish, insects and polychaetes. The longterm ecological significance of such selection is not known. Loss of genetic diversity may lead to increased inbreeding and associated massive declines in fertility and offspring viability, as the exposed population becomes increasingly homozygous, allowing the expression of detrimental recessive genes. Thus genotoxicity can result in rapid alterations in gene frequencies (relative to normal evolutionary rates) in natural populations, the ecological consequences of which are poorly understood but are likely to be serious.

**Ecological implications of genotoxic effects.**

The aim of genetic ecotoxicological research on marine biota is to determine whether anthropogenic chemicals and radiation are able to damage DNA sufficiently to alter the population dynamics and community structure of biota in ecosystems and to identify trans generational effects, that may be of special significance. It is important therefore, that researchers move beyond gathering evidence of exposure to genotoxins by relating genetic damage to changes in phenotypic attributes that influence growth and reproductive success. It is surprising how little work is done in this area.

Although it is apparent that man-made chemicals are causing major concern in environmental pollution, natural agents are also not exempt from carcinogenic, mutagenic and teratgenic hazards. (Payne and Rahimtula, 1989). Polyhalogenated compounds isolated from a marine algaewere found to be mutagenic, one of them being 200 times more potent than a typical synthetic mutagen and carcinogen, i.e., EMS (ethyl methane sulfonate) (Leary et al. 1979). It has been evaluated that the global production of the mutagen and carcinogen methyl iodide may exceed industrial pollution by a factor of 80 (Payne and Rahimatula, 1989). Marine algae may be a significant source of methyl chloride, which is the principal halocarbon in the atmosphere. (Zafiriou 1975).

It is virtually impossible to compile a list of genotoxicants in the marine environment. In general, the assessment is extremely difficult and often controversial from a scientific point of view. Chemical families generally include a broad variety of structurally related compounds, whose differential toxicological impact is hardly predictable. GESAMP( Group of Experts on Scientific Aspects of Marine Pollution) identified as many as 800 chlorinated hydrocarbons having relevance to the marine environment. (GESAMP. 1990). Tentative list of carcinogens in the marine environment have been

prepared using the data base available in the IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans. Most important potential marine pollutants were reported to be polychlorinated biphenyls, cadmium and cadmium compounds, polycyclic aromatic hydrocarbons etc. Acrylamide could occur in water when polyacrylamides are used in drilling operations. Pesticides such as lindane, DDt, mirex and toxaphene can accumulate in fish. The chlorophenols, particularly pentachlorophenol, can be found in biota and could occur as marine pollutants. (De flora et al. 1991). A broad literature is available on the presence of petroleum hydrocarbons in marine ecosystems. Halogenated hydrocarbons can contaminate the marine environment through agricultural runoff, rivers and discharge of industrial and municipal wastes. Metals can be released into the marine environment from both natural and anthropogenic sources.

Paradoxically even substances used to lessen marine pollution may possess genotoxic properties. A case in point is oil dispersants used as antidotes for oil spills. For instance an oil dispersant caused spinal deformities in hatched larvae of sea bass (Dicentarchus labrax) ( De Flora 1991). Another important example is nitrilotriacetic acid (NTA), which has been proposed and used, often in controlled amounts, as a substitute for polyphosphates in household laundry detergents. As such it is a valuable tool for preventing seawater eutrophication, but it is also a massive contaminant of marine bodies receiving domestic sewage effluents.

A variety of putative inhibitors of mutagenesis and carcinogenesis have been isolated from the marine environment. More than 20 thiol compounds were found to occur in sediment pore-water samples in Florida. These organosulfur compounds are among the most promising protective agents, displaying antimutagenic and anticarcinogenic mechanisms. Unfortunately, protective factors can quite often behave like double-edge swords . Thus essential nutrients having a well-known protective role can become a cause of disease and vice versa, typical pollutants can act as inhibitors of disease development. An example of former situation is provided by selenium. An example of typically hazardous marine pollutants which may protect from cancer is provided by polychlorinated biphenyls (PCBs), which are potent inducers of mixed function oxidases not only in mammals but also in fish (De flora et al. 1991).

**Strategy for assessment of genotoxic effects**

Assessment of exposures of organisms to genotoxicants in the aquatic environment is complicated. There are multiple potential pollutants often encountered as complex mixtures. Marked seasonal variations occur in for eg, environmental conditions, diet and hormonal status. These factors can have a dramatic influence on the enzyme systems that activate and detoxify genotoxicants. Further-more many aquatic organisms are not sedentary, complicating any biomonitoring programme.. In the marine environment, the situation is further complicated. Here we are presented with unique challenges in the determination of environmental injury compared to scientists studying terrestrial or freshwater ecosystems. 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 difficult.

Harmful substances tend to undergo interactions and transformation in sea water, sediments and marine biota, due to physical, chemical, microbial, or light mediated mechanisms. Bioaccumulation phenomena in marine organisms may result from food-chain biomagnification processes or from concentration of pollutants by filter feeders. The most extensively investigated example of this type is inorganic mercury, which is methylated by a variety of marine bacterial and fungal species to yield methyl mercury. Bioaccumulated organic mercury is more toxic than inorganic mercury to higher organisms, including humans. Mercury methylation by sediment bacteria increases its lipophilicity and accumulation in fish (Hodson 1988). Metabolic transformations of xenobiotics occur in all marine organisms, the biochemical mechanisms in fish being comparable to those extensively investigated in mammals. Induction of metabolic pathways, and especially of the mixed-function oxygenase system, represents the earliest

warning signal of exposure to pollutants. Occurrence of neoplastic diseases is documented by experimental and field studies in marine vertebrates as well as in invertebrates. Pollutants of the marine environment undergo a large variety of interactions with other pollutants as well as with normal chemical components of the contaminated ecosystem. The outcome of chemical interactions, often with an extremely high number of combination keys, is hardly predictable in the majority of cases. Several interactions occurring in the marine water and biota bear relevance to carcinogenic, mutagenic and teratogenic problems.

One means of detecting and monitoring the exposure of organism to genotoxins is to measure the degree of induction of protective enzyme systems and DNA repair system. This is known as the biomarker approach (McCarthy and Shugart 1990). Nunn et al. 1996 reviewed studies in which biomarkers have been used to monitor exposure to genotoxins in marine organisms in situ. Salmonella/microsome test and microsreen phage induction assays were also employed for testing genotoxic activity of Heavy metal and organic contaminated water and sediment samples( Vargas et al. 2001). A cytogenetic approach using the Cytokinesis blocked micronucleus assay in culured human lymphocytes to evaluate water quality has also been reported (Lemos and Erdtmann 2000). In fish at least gametes are approximately 1500 times more susceptible than adults to genotoxicity associated with exposure to ionizing radiation. Whether the same is true regarding chemical genotoxins awaits investigation.

**Genotoxicity of tributyltin(TBT) compounds**

Tributyltin compounds, used in antifouling paints are considered to be one of the most toxic anthropogenic substances to enter the marine environment. The effects of leachate from antifouling paints on marine organisms first became apparent in the late 1970s when it was suspected to be the cause of declining oyster production in France and subsequently in different parts of the world (Alzieu, 1991). This led to the implementation of legislation in Europe and North America to restrict the use of TBT-based paints on small boats(<25 m in length), and in aquaculture. However, TBT from larger vessels, excluded from the ban, is also known to have an impact on marine populations and remains an environmental problem of concern in different parts of the world (Mogan et al., 1998). The most dramatic effect of TBT has been irreversible sexual abnormality in female neogastropod snails. This phenomenon known as "imposex", is a masculinisation process involving the development of male sex organs in females due to hormonal imbalance. This hormonal imbalance leading to imposex formation prevents normal breeding activity, ultimately causing population disappearence.

Adapting an integrated approach the potential genotoxic, cytotoxic and developmental effects of tributyltin oxide(TBTO), a known endocrine –disrupting agent for neogastropods, had been evaluated in two ecologically relevant invertebrates: target species, Mytilus edulis (blue mussel) and non-target species Platynereis dumerilli (rag worm). The study suggested that tributyltin is capable of inducing cytogenetic damage, sister chromatid exchanges (SCE) and chromosomal aberrations (CA) in this target organisms. The chromosome complement of Medulis (2n =28) facilitated the analysis of both CAs and SCEs. The study emphasizes the need for investigations of other endocrine disrupters on ecologically relevant aquatic invertebrates, which contribute to the maintenance of ecosystems and that could potentially be harmful to human health via the food chain (Jha et al. 2000 a,b). A workshop to formulate the research strategy for investigating the ecological significance of endocrine disruption, has recommended P. dumerilli as a sentinel annelid species, which plays an important role in the marine ecosystem (Taylor et al. 1999).

**Genotoxic evaluations in marine organisms.**

Cytogenetic techniques have already been applied for monitoring fish or mussel exposures to pollutants under field conditions or for assessing water quality under lab conditions. A number of laboratory studies aimed at developing and validating cytogenetic techniques in aquatic organisms exposed to genotoxicants have been reported (Hyashi et al. 1998). Cytogenetic analyses can detect gross genomic

alterations after exposure to genotoxicants. In spite of certain technical difficulties, the assessment of chromosomal aberrations, (CA) sister chromatid exchanges (SCE) and micronuclei (MN) has also been applied to marine vertebrates and invertebrates(Ayllon et.al 2000).

Fish micronucleus assays have been shown to be usefull in vivo technique for in situ monitoring of water quality. Al Sabti and Metcalfe 1995 reviewed the literature on the clastogenic effects of chemical and physical agents on fish cells with emphasis on the induction of micronucleus in teleosts. This review is directed at assisting laboratories in the development of fish genotoxicity assays for water quality monitoring.

Bivalve mussels (Mytilus edulis and Mytilus galloprovincialis) seem to be ideal bioindicators of exposure due to their ability to concentrate local pollutants. MN and SCE frequency in the gill tissue and developing eggs of M.galloprovincialis and CA and SCE frequency in gill tissue of M.edulis have been reported(Jha et al.2000). In fact only a low fraction of the gill cell population undergoes mitosis as assessed both in mussels and fish species. Developing eggs in mussels are quite an appropriate target for SCE induction, because they contain a population of actively proliferating cells. Use of antikinetochore antibody allowed the distinction between micronuclei resulting from acentric fragments and those from lagging chromosomes in M.galloprovincialis (De Flora et al. 1991).

Other experimental studies have investigated cytogentic aberrations in various fish tissues. For instance CA in various tissues of Boleophthalmus dussumieri (Krishnaja and Rege 1982) CA and SCE in the haemopioetic tissue (van der Gaag and van der kerkhoff 1985, Maddock et al. 1986). Induction of CA was observed in fish eggs and larvae collected from polluted areas of the Atlantic U.S. coast (Longwell and Hughes 1980). Cytogenetic changes in polychaetes were studied and Neanthes arenaceodentata was proposed as a model for marine genetic toxicology as early as 1980.

Michelmore and Chipman 1998 reported the use of DNA strand breakage as an endpoint for environmental monitoring in aquatic organisms. Studies with aquatic organisms exposed to polluted waters or sediments in the field supported the use of the comet assay and other DNA strand breakage methods as rapid sensitive screens for genotoxic pollutant exposure.

A target organism of special interest is the sea urchin. Cytogenetic alterations produced by genotoxicants in treated embryos or in embryos following adult or gamete exposure are one of the various sublethal end points that can be monitored in these cosmopolitan metazoan systems. Mitotic aberrations in sea urchins include stray chromosomes, attached fragments, bridges, multipolar spindles and acentric fragments. Induction of MN has also been reported following exposure of the purple sea urchin to environmental levels of benzopyrene.(De Flora 1991).

**Large scale genotoxicity assessments in the marine environment**

There are a number of techniques for detecting genotoxicity in the marine environment. Many of these are applicable to large scale field assessments. Certain tests concentrate on genotoxic evaluation in target organisms in situ. Others utilise 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 endpoint, 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 (Hose and Brown 1998). The anaphase aberration test is independent of karyotype. The only requirement is to have sufficient mitotic cells for analysis and can be performed with rapidly growing early life stages. When used with indigenous species the anaphase aberration test is amenable for evaluation of genetic effects in commercially and ecologically important fishes, many of which are difficult to spawn in the laboratory. The anaphase aberration test can also be used with preserved samples so archived ichthyoplankton

samples often routinely collected as part of monitoring programmes can be used to establish baseline values. The anaphase aberration test is particularly suitable for examining the potential genotoxicity of poorly characterized complex mixtures or polluted waters.

Efficient coastal management requires systematic monitoring network of harmful pollutants in the marine waters, sediment and biota. It is high time we identified target and nontarget (surrogate) species indigenous to our marine waters for genotoxicity evaluation. The bivalve mussel, Mytilus edulis is a promising candidate. The development of genotoxicity assay systems that use aquatic vertebrates and invertebrates to assess genotoxicity of water in the field conditions and laboratory is also urgently warranted.

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