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Review Article

FISH ECOGENOTOXICOLOGY: AN EMERGING SCIENCE, AN EMERGING TOOL FOR ENVIRONMENTAL MONITORING AND RISK ASSESSMENT

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ABSTRACT
Ecogenotoxicology (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. Contrary to human toxicology studies which focus on the fate of the individual, ecogenotoxicology evaluates the consequences of genotoxicants for population sizes and structure, but applies the principles of genetic toxicology in hazard and risk assessment. Genetic hazard assessment, thus, deals with changes in genetic material of organisms, either human or other natural origin. Several reviews demonstrate the presence and potency of genotoxins from a broad range of industrial and municipal effluents. There is a close association of DNA damage, mutation, and induction of various types of cancer. Fish serves as useful genetic model for the evaluation of pollution in aquatic ecosystems. Fish species from contaminated areas initiated studies in the aquatic environment and evidence is growing that environmental mutagens can reduce the reproductive success of populations. Different genotoxicity tests and their applications to environmental monitoring and assessment have been variously reported in fish. This review paper, thus, examines the use of ecogenotoxicology in environmental monitoring, the role of fish in genotoxicity testing of pollutants, genetic basis in genotoxicological assessment, current methods of ecogenotoxicological hazard assessment using fish in vitro and in vivo, and their applications to environmental monitoring as well as recent advances in the field of fish ecogenotoxicology. Limitations and recommendations for further research on the use of ecogenotoxicology was also highlighted.

KEY WORDS: Ecogenotoxicology, Environmental monitoring, risk assessment, DNA damage, Genotoxicity tests.

INTRODUCTION

Pollution of the environment has become a major concern of society (Shugart and Theodorakis, 1998). One of the most sensitive concerns is the potential for exposure to substances that are genotoxic . A genotoxic chemical or physical agent has the ability to induce mutations or so called indicator effects which are mechanistically associated with the formation of mutations (for example, induction of DNA modifications, DNA repair, or recombination) (Belfiore, 1998).

Environmental contaminants can affect the genetic makeup of populations in three ways: via mutations, genetic drift, and genetic adaptation (Belfiore, 1998). Some of these pollutants are carcinogenic and mutagenic with the capacity to affect both the structural integrity of DNA and the fidelity of its biological expressions (Wogan and Gorelick, 1985).

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 effects on the health of the organism (Shugart and Theodorakis, 1998). Ecogenotoxicology (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 (Shugart and Theodorakis, 1998). Genetic hazard assessment, thus, deals with changes in genetic material of organisms, either human or other natural origin (OSPAR, 2002). Several review demonstrate the presence and potency of genotoxins from a broad range of industrial and municipal effluents (De Raat *et al.,* 1990; White *et al.,* 1996a; Claxton *et al.,* 1998) as cited by OSPAR, 2002. There is a close association of DNA damage, mutation, and induction of various types of cancer (OSPAR, 2002). Fish serves as useful genetic model for the evaluation of pollution in aquatic ecosystems (Mitchell and Kennedy, 1992; Park *et al.,* 1993). Fish species from contaminated areas initiated studies in the aquatic environment (Murchelano and Wolke, 1991; Mc Mahon, 1994; Moore and Myers, 1994) and evidence is growing that environmental mutagens can reduce the reproductive success of populations (Anderson and Wild, 1994). Different genotoxicity tests and their applications to environmental monitoring and assessment have been variously reported in fish (Hartmann *et al.,* 1999; Gartiser *et al.*, 2001; White *et al.,* 1998a; White *et al.,* 1998b; Helma *et al*., 1996; Vargas *et al*., 2001; Hose and Brown, 1998; Stahl, 1991; Mitchelmore and Chipman, 1998b; Grummt, 2000b). Some of the methods are based on OECD and EC guidelines used for chemical risk assessment (OSPAR, 2002).

Genetic mechanism of changes in ecogenotoxicology

One of the crucial questions in the field of environmental genotoxicology is how the potential hazards and risk of genotoxic substances should be evaluated (Roex *et al*., 2001). To answer this question a distinction has to be

made between the different pathways along which a chemical is able to affect the genetic structure of an organism and the subsequent effects this may have for the populations in the field (Roex *et al*., 2001).

It is difficult to demonstrate the effect of environmental stressors, including genotoxicants, at the ecosystem level, where population and communities are studied because the responses observed are latent and so far removed from the initial event(s) of exposure that causality is often almost impossible to establish (Shugart and Theodorakis, 1998). A way to solve this problem is to view ecosystem as dynamic interactions of living and inert matter where the living material acclimates and adapts to environmental changes. These processes are physiological and have genetic basis, therefore, understanding changes at the genetic level (DNA) should help define the more complex changes at the ecosystem level (Shugart and Theodorakis, 1998).

The genetic apparatus of an organism can interact with genotoxicants in a variety of ways and an understanding of the cellular mechanisms involved in these interactions provide the researcher the opportunity to predict and possibly prevent contaminant-induced genetic damage in exposed populations (Shugart and Theodorakis, 1998). Genotoxicants can alter the structural integrity of the DNA, cause mutations and subsequent heritable effects or even cause non-mutagenic effects. Conversely, the organism may perceive the genotoxicant and attempt to eliminate the agent or repair changes to the DNA (Guengerich, 1993). If the genotoxic agent directly attacks the DNA, the organism may perceive this damage and attempt repair (Shugart *et al.*, 1992). The flow of genotoxic stress within a somatic cell (Brusick, 1980) and the mechanisms involved have been reviewed (Thilly and Call, 1986; Clive, 1987). Cellular processes regulating these events in the DNA are very complex and for which there do little understand (Shugart and Theodorakis, 1998). These processes are affected differently in different species and may depend upon, for example, the type or class of genotoxic agent and the reactivity of its metabolites, capacity of the cell to recognize and suppress the multiplication of cells with aberrant properties (Clive, 1987). Effects expressed in somatic cells can be detrimental to the exposed individual, whereas, mutational events may affect subsequent generations (Shugart and Theodorakis, 1998). Extrapolation of effects on somatic cells to germ cell level of organization is difficult due to the inherent difference in sensitivity of these type of cells to genotoxicants (Wurgler and Kramers, 1992). Furthermore, establishing a causal relationship between a genotoxic agent in the environment and a deleterious effect in subsequent generations of that organism is also highly unlikely because individuals carrying harmful mutations are eliminated from the population due to a strong selection against less fit and less well-adapted individuals (Bickham and Smolen, 1994).

Role of fish in Ecogenotoxicology

Genotoxins are chemicals are responsible for DNA damage in variety of aquatic organisms and fishes in particular causing malignancies, reduced growth, abnormal development, reduced survival of embryos, larvae, and adults, ultimately affecting the economy of fish production significantly. Genotoxicity not only reduces the "fitness" in wild fish populations, but also pose risk to human health via food chain (Kapour and Nagpure, 2005). Although, technical advancements have been made in some mammalian species and also in fruit flies, the desired progress has not been achieved towards evaluation of potential hazards and risks from genotoxic pollutants in fish species (Kapour and Nagpure, 2005).

The selection of fish as a model in ecogenotoxicological studies could be made necessary since fish is a very sensitive biomarker indicator of water quality and can highlight the potential danger of new chemicals introduced in the aquatic environment (Bailey *et al*., 1992) and also respond to toxicants in a manner similar to higher vertebrates (Al-Sabti and Metcalfe, 1995). Fish serves as useful genetic model for the evaluation of pollution in aquatic environment (Mitchell and Kennedy, 1992; Park *et al*., 1993). Current awareness of the potential hazards of pollutants in the aquatic environment has stimulated much interest in the use of fish as indicators for monitoring carcinogens, teratogens, clastogens, and mutagens (Obiakor *et al.,* 2012). This is because aquatic environment serves as convenient repositories for man's biological and technological wastes (Cajaraville *et al*., 2000). Fish play different roles in the trophic web such as undergoing bioaccumulation of environmental pollutants and biotransformation of xenobiotics through cytochrome p450-dependent oxidative metabolism; besides they respond to mutagens at low concentrations (Goksoyr *et al*., 1991). Fish cells retain important traits of fish; for example, poikilothermic behaviour, unique xenobiotic metabolism, and low rate of repair mechanism; they have been shown to be more sensitive for the induction of genetic damage (Kapour and Nagpure, 2005). DNA repair has been shown to be slower in fishes than mammals (Walton *et al*., 1984; Espina and Wesis, 1995). Therefore, they can be used as sentinel organism for biomonitoring studies (Landolt and Kocan, 1983). Fish have severally been used in several eukaryotic genotoxicity and mutagenicity tests, which include its use in Comet assay (Sumathi *et al*., 2001), DNA repair synthesis (Mullerschon, 1989; Grummt, 2000b), Chromosomal aberration test (Al-Sabti, 1985; Rishi and Grewal, 1995), Micronucleus assay (De Flora *et al*., 1993; Saotome and Hayashi, 2003; Pantaleao *et al*., 2006), and Sister chromatid exchange test (Kligerman *et al*., 1984; Sahoo *et al*., 1998). Therefore, efforts should be made to utilize assays for detecting genotoxicity caused by aquatic pollutants in fishes at DNA level. This will help in formulating long-term strategies for fish conservation programme besides estimating safe Level of pollutants in water (Kapour and Nagpure, 2005).

Role of ecogenotoxicology in environmental monitoring

Contrary to human toxicology studies which focus on the fate of the individual, ecogenotoxicology evaluates the consequences of genotoxicants for population sizes and structure. Investigations showing high prevalence of hepatic tumors in different fish species from contaminated areas initiated studies in the aquatic environment (Murchelano and Wolke, 1991; McMahon, 1994; Moore and Myers, 1994). Several examples of neoplasms in fish due to waste water effluents have been described

(Metcalfe and Sonstegard, 1985; Kimura *et al*., 1989). Exposure to DNA-damaging agents may result in the formation of carcinogen-DNA adducts, which, as possible indicators for carcinogens, have been detected in mussels (Harvey *et al*., 1997) and fish from contaminated sites (Dunn, 1991; Weishburger and Williams, 1991; El Adlouni *et al*., 1995; Erickson and Larsson, 2000). Thus, detection of adducts provide a way of documenting exposure. This approach was used to examine DNA from beluga whales in St Lawrence estuary, Quebec, Canada, to determine whether exposure to benzo (a) pyrene (BaP), a potent environmental carcinogen and the suspected etiological agent for the high incidence of cancer in these animals had occurred (Martineau *et al*., 1988).

Early in 1987 (Shugart and Theodorakis, 1998), the detection of excessive strand breakage in the DNA of several aquatic species was implemented as a biomonitor for environmental genotoxicity as part of the Biological and Monitoring and Abatement Program for the US Department of Energy (USDOE) Reservation in Oak Ridge, Tennessee. This approach was effectively used in studies with two species of turtles, the snapping turtle (*Chelydra serpentine*) and Pond slider (*Trachemis scripta*) (Meyer-Schone *et al*., 1993) using the Alkaline DNA unwinding assay (Shugart, 1998). Similarly, analysis of strand breaks in Sun fish (Shugart and Theodorakis, 1998), using the DNA alkaline unwinding assay (Shugart, 1998), has been employed as a biological marker for environmental genotoxicity as part of the Biological Monitoring and Abatement Program at East Fork Popler Creek (Shugart, 1990). This creek is the receiving stream for industrial effluent from the USDOE reservation in Oak Ridge, Tennessee, USA. Water and sediments downstream contain metal, organic chemicals and radionuclides discharged over many years of operations (Shugart, 1990). The erythrocyte micronucleus test has been used with different fish species (Obiakor *et al*., 2012) and other marine shellfish to monitor aquatic pollutants displaying mutagenic features in developed countries (De Flora *et al.,* 1993; Saotome and Hayashi, 2003; Pantaleao *et al.*, 2006). Current awareness of the potential hazards of pollutants in the aquatic environment has stimulated much interest in the use of fish as indicators for monitoring carcinogens, teratogens, clastogens and mutagens (Obiakor *et al*., 2012). This is because aquatic environment serves as convenient repositories for man's biological and technological wastes (Cajaraville *et al*., 2000). Aquatic animals have often been used as assay to evaluate surface water (Brugs *et al.*, 1977, Carins *et al.,* 1975). Substances displaying mutagenic, teratogenic and carcinogenic potentials are easily evaluated because of high sensitivity of these organisms to these pollutants at low concentrations (Koeman *et al.,* 1977, Poele and Strik, 1975). Obiakor *et al.* (2010a) and Obiakor *et al*. (2010c) have demonstrated the use of *Synodontis clarias* and *Tilapia nilotica* from freshwater of the Anambra River, Nigeria, in ecogenotoxicology studies using the micronucleus test and validating them as index of cytogenetic damage, monitoring of aquatic genotoxicants and other sublethal concentrations of chemical pollutants.

Ideally, genetic ecotoxicology will begin to address such outcomes of exposure to environmental genotoxicants as disease, decreased reproductive success, and altered genotypic diversity (Shugart and Theodorakis, 1998) using endpoints such as frequencies of gametes loss due to cell death, embryo mortality caused by lethal mutations, abnormal development, cancer, and mutation frequencies affecting the gene pool of exposed populations (Anderson and Wild, 1994). But, up till now only endpoints like gamete loss or teratogenic effects as well as cancer incidences can be measured (OSPAR, 2002). Effects for exposed populations might be estimated in case where these populations are ecologically characterized, but knowledge about consequences of genotoxic exposure on the gene pool of exposed species is still scarce (Theodorakis and Shugart, 1998; OSPAR, 2002), however, the principles underlying research of effects of genotoxicants on genetic diversity are not new as there are newer approaches to describe genetic effects of contaminants on the population level (Bicham and Smolen, 1994; Anderson *et al.,* 1994; Roex *et al*., 2001), which focus on the genetic diversity, examining the current status and history of population by molecular genetic technique (Shugart and Theodorakis, 1998). But these effects are not necessarily caused by mutagenicity; they depend also on chronic effects and population size (Bickham *et al.*, 2000).

In a heterozygous population, there are likely to be certain genotypes that are more sensitive to genotoxic exposure than others. This is so if the population is heterozygous at loci that are both critical to fitness and susceptible to toxicant-induced structural alterations (Shugart and Theodorakis, 1998). Genotoxic exposure can act as a selective force by eliminating sensitive genotypes, or reducing the number of offspring that they contribute to the next generation. The result can be a reduction in the total genetic variation within the population or a shift in genotypic frequencies (Shugart and Theodorakis, 1998).

Role of ecogenotoxicology in environmental risk assessment

Genetic hazard assessment investigates changes in genetic material of organisms, either human or other natural origin (OSPAR, 2002). A review of ecogenotoxicology in environmental risk assessment has been presented by Roex *et al*. (2001). Regulatory authorities worldwide require data on the genotoxic potentials of new drugs and chemicals (Jena *et al*., 2001) through genotoxicity testing for hazard identification with respect to DNA damage (Madle *et al*., 1987) and biological information indicative of toxicity, which can be interpreted and/ or extended to the assessment of health risk to humans (Nath and Krishna, 1998) and the environment (Roex *et al*., 2001). Today, in the pharmaceutical industry, it is not possible to register a new drug without providing information on its mutagenicity (Cartwright and Mathews, 1994). In ecogenotoxicology, possible effects of mutagenic/ genotoxic substances on populations and ecosystems are investigated. Mutagenicity testing of genotoxic substances has been performed with all types of organisms (OSPAR, 2002).

In risk assessment of chemicals, a first screening for mutagenicity takes place in a battery of three *in vitro* (*in situ*) genotoxicity test, after which an *in vitro* carcinogenicity test is carried out based on a position

result in the *in vitro* test (Kramer *et al*., 1992), the result of which is extrapolated to carcinogenic risk for humans by calculating a lifetime exposure level corresponding to a unit risk of 10^{-6}, which is accomplished by linear extrapolation from lowest effective dose to 0 (Roex *et al*., 2001). Ecological risk assessment concerns a wider range of species instead of a single one like in human genotoxicology, and has to deal with the protection of populations instead of individuals (Mohn and De Raat, 1983; Wurgler and Kramers, 1992).

Test animals that are used in carcinogenicity studies for risk assessment are mostly mice, rats, or hamster for which extrapolation to human situations makes them suitable models (Roex *et al*., 2001). However, for extrapolation to ecosystem, carcinogenicity test batteries with more representative species such as fish, daphnia, and algae used in ecological risk assessment procedures are appropriate as these models, particularly fish, have been used severally in ecological risk assessment studies (Amanuma *et al*., 2000; Burhart, 2000) demonstrating the ecogenotoxicological significance of these models.

Applications of ecogenotoxicological methods in monitoring and risk assessment

For monitoring purpose, higher organisms (eukaryotes) are exposed to environmental compartment "in situ" or in laboratory test "in vivo" (OSPAR 2002). Some of the methods applied to environmental samples are based on corresponding OECD and EC guidelines used for chemical risk assessment, but others have not yet been standardized (OSPAR, 2002). The bacterial Ames test (Ames *et al*., 1973), Umu-C assay (Oda *et al*., 1985), and SOS chromo assay (Quillardet *et al.,* 1982; 1985) have been applied predominantly to waste water samples. Tests with eukaryotes cells or organisms are relevant for ecological risk assessment-plants, amphibians, fish, permanent cell lines such as Chinese hamster lung cells (V79) (Gartiser and Brinker, 1996; Gartiser *et al*., 1996; Jager *et al*., 1996a; Miltenburger, 1997), Chinese hamster ovary cells (CHO) (Strniste *et al*., 1982; Waters *et al*., 1989; Venegas and Garcia, 1994), and Chinese hamster lung cells (CHL) (Nobukawa and Sanukida, 2000), marine and freshwater mussels-have been used as test organisms (OSPAR, 2002). An overview of some genotoxicity test methods and their application to monitoring and assessment is given below.

Comet assay

The comet assay has been developed from the method of Rydbert and Johansen (1978), who were the first to perform a quantitation of DNA damage in single cells. Later on, Ostling and Johanson (1984) improved the assay by developing an electrophoretic microgel technique under neutral conditions and stained the DNA with acridine orange. The more versatile alkaline method of the comet assay was developed by Singh *et al*. (1988), which was developed to measure low levels of strand breaks with high sensitivity. In general, cells are mixed with low-melting agarose placed on microscope slides and lysed by an alkaline buffer with ionic detergents. The liberated DNA is resolved in an electrophoresis chamber, stained and evaluated by florescence microscopy. Cells with increased DNA damage display increased migration from the nuclear region towards the anode (Singh *et al*., 1988). The resulting comet like structure is quantified by measuring the length of the tail and/ or tail moment (the intensity of the migrated DNA multiplied by the respective tail length with respect to the DNA). A review of th eaplicability of the comet assay in environmental monitoring has been provided by Mitchelmore and Chipman (1998b) and has been applied to a broad range of aquatic organisms, including fish (Pandrangi *et al*., 1995; Devaux *et al*., 1997; Belpaeme *et al*., 1998; Risso-de Faverney *et al*., 2001).

DNA Alkaline unwinding assay

The level of DNA strand breaks with respect to the total DNA can be determined by following a time-dependent alkaline unwinding assay. Unwinding of DNA takes place at single stranded breaks, hence the amount of double stranded DNA remaining after a given period of alkaline exposure will be inversely proportional to the number of strand breaks; this ratio is expressed in form of F values, which measures the relative double strandedness of a particular DNA (Shugart, 1998). *In situ* investigations for for the detection of genotoxic potential in selected surface water with the DNA alkaline unwinding assay have been reported using fish cells, early life stages of fish, crustaceae, and mussels (Meyers-Schone *et al.*, 1993; Wittekindt *et al*., 2000). Everaarts and Sarkar (1996) studied DNA damage in sea stars (*Asterias rubens*) in order to assess the state of pollution of the North Sea.

DNA repair synthesis (UDS-assay)

The unscheduled DNA synthesis assays measures the incorporation of radioactively labelled nucleosides (usually tritium-labelled thymidine) in cells that are not undergoing scheduled DNA synthesis. The DNA repair synthesis UDS test has been applied using primary hepatocytes from fish to assess genotoxicity in surface water (Mullerschon, 1989; Grummt, 2000b).

Chromosome aberration test

Chromosome mutation is a macrodamage of chromosome (OSPAR, 2002). Chromosome aberration includes structural aberrations such as fragments, intercalations, and numeral aberrations resulting from either direct DNA breakage or inhibition of DNA synthesis (Nagpure *et al*., 2005). Cytogenic effects can be studied either in whole animals (*in vivo*) or in cells grown in culture (*in vitro*) (Nagpure *et al*., 2005). Generally, the cell culture is exposed to the test substance and then afterwards treated with a metaphase-arresting Colcimide (OSPAR, 2002) or Colchicine (Nagpure *et al*., 2005). Following suitable staining the metaphase cells are analysed microscopically for the presence of aberration.

Although, cytogenic studies were initiated by Retzius (1890) on agnathan (*Myxine gluttinosa*), fish cytogenetics got real momentumwith the work of Mekino (1934) as cited by (Nagpure *et al*., 2005). Since then, the test has been carried or evaluated in several fish species (Rishi and Grewal, 1995; Al-Sabti, 1985; Arockia and Selvanayagan, 1998; Anitha *et al*., 2000).

Micronucleus assay

The micronuclei are chromosome fragments or whole chromosomes that were not incorporated in the daughter cell nuclei and appear in the cytoplasm (Schmid, 1975). The micronucleus test is a simple and sensitive assay for "in vivo" evaluation of genotoxic properties of various agents. Chromosomes in fish cells are usually of small size

and occur in large numbers; therefore, it can be easily applied to fish or other aquatic organisms sine small and large number of chromosome do not affect the micronucleus assay (Al-Sabti and Metcalfe, 1995).

Environmental biomonitoring with micronucleus assays usually has been performed "in vivo" by exposure of relevant aquatic organisms for several days followed by microscopic analysis of erythrocytes, gill cells. But permanent fish cell lines (RTG-2) have also been used "in vitro" (Chung *et al*., 1997; Kohlpoth *et al*., 1999). ‘‘In vivo" studies with fish have severally been used and reported for genotoxicity with the micronucleus (Odeigah and Osaneyinpeju, 1995; Tuviene *et al*., 1999; Obiakor *et al*., 2012).

Sister chromatid exchange (SCE) test

The sister chromatid exchange test detects reciprocal exchanges of DNA segments between two sister chromatids of a duplicating chromosome (Kumar *et al*., 2005). Although little is known about the molecular basis, the SCE frequency is elevated under the influence of mutagenic agents and therefore serves as a model for genotoxicity (OSPAR, 2002; Ravindra *et al*., 2005). For genotoxicity assessment in environmental samples SCE assays have been performed with mussels (Jha *et al*., 2000a; 2000b), fish cells (Kligerman *et al*., 1984; Zakour *et al*., 1984; Sahoo *et al*., 1998).

Recent developments

In the field of genotoxicological evaluation of environmental samples, recent advancement has been achieved (OSPAR, 2002). Amanuma established a transgenic zebrafish for the detection of mutagens; it carries plasmids that contain the rpSL gene of *Escherichia coli* as a mutational target gene (Amanuma *et al*., 2000). Winn *et al*. (2000) prepared a transgenic fish that carries multiple copies of a bacteriophage lambda vector that harbours the cII gene as a mutational target, a technique originally developed for lambda transgenic rodents. The p53 tumor suppressor gene, which is known to be implicated in cancer development, has been investigated as a possible biomarker for genotoxin in fish cells (McMahon, 1994; Bhaskaran *et al*., 1999; 2000). The amplification of DNA by polymerase chain reaction technique enabled the detection of mutations at specific sites and the development of electrochemical DNA based biosensors (Kennerley and Parry, 1994; Mascini *et al*., 2001).

Limitations in ecogenotoxicology

Increased mutations rates due to environmental pollution might negatively affect populations (OSPAR, 2002). This is still controversially debated in the scientific community (Wurgler and Kramer, 1992; Anderson and Wild, 1994) but evidence is growing that environmental mutagens can reduce reproductive success of populations (OSPAR, 2002). Even though an increasing number of studies involving ecogenotoxicity are available (Hose and Brown, 1998; Hutchenson *et al*., 1998; Theodorakis *et al*., 1998; Rodgers and Baker, 2000), the identification of clear cause-effect relations is increasingly complicated, the higher the level of biological organization. For instance, For example, Shugart and Theodorakis (1994, 1996) examined a series of retention ponds heavily contaminated with radionuclides, but which support a resident population of mosquitofish (*Gambusia affinis)* for the past 20 years. They reported that there was an inverse correlation between DNA strand breakage and fecundity of fish from the contaminated ponds (Shugart and Theodorakis, 1998). This has implications for higher-order ecological 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 (Shugart and Theodorakis, 1998). 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 which display these banding patterns at one of the contaminated sites have a higher fecundity and lower degree of strand breakage than do 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 between genotype, DNA strand breakage, and fecundity.

Ideally, genetic ecotoxicology will begin to address such outcomes of exposure to environmental genotoxicants as disease, decreased reproductive success, and altered genotypic diversity (Shugart and Theodorakis, 1998) using endpoints such as frequencies of gametes loss due to cell death, embryo mortality caused by lethal mutations, abnormal development, cancer, and mutation frequencies affecting the gene pool of exposed populations (Anderson and Wild, 1994). But, up till now only endpoints like gamete loss or teratogenic effects as well as cancer incidences can be measured (OSPAR, 2002). Effects for exposed populations might be estimated in case where these populations are ecologically characterized, but knowledge about consequences of genotoxic exposure on the gene pool of exposed species is still scarce (Theodorakis and Shugart, 1998; OSPAR, 2002). Majority of the currently used genotoxicity testing assays for regulatory toxicity testing were developed in the 1970's (Jena *et al*., 2001). In most of the cases, the site and mechanism by which genotoxicity is produced by the compound under the study is not known (Jena *et al*., 2001). It may happen that the target site of toxic action may not be the same target site of toxic action of a new chemical entity (NCE) (Jena *et al*., 2001). Also, In subchronic and chronic toxicity testing, several pertinent parameters or endpoints can be detected to determine the toxicity, but the same is rarely true for genotoxicity tests (Nath and Krishna, 1998). Moreover, for certain categories of chemicals (Jena *et al*., 2001), which need critical experimental evaluation, there are no details with regards to the choice of specific test system and test protocols

(Muller *et al*., 1991). Most guidelines are devoid of recommendations for compounds, which are genotoxic, but seem to act by non-DNA target (Tennant *et al*., 1987). There are also no specific recommendations on the threshold of different genotoxic and tumorogenic compounds and their organ-specific effects when they are intended to use therapeutically (Scott *et al*., 1991). A single test system cannot be designed for universal detection of the relevant genotoxic substances; testing requirements depend on the nature and category of chemical substances (Jena *et al.*, 2001). In addition, there is no validated test system for detecting induced genome mutation (aneuploidy) in germ cells (Allen *et al.,* 1986).

CONCLUSION

It is now clear that environmental genotoxicology holds the key to early detection and monitoring of pollution in aquatic environments, particularly when fish species are the test organisms. Fish serves as useful genetic model for the evaluation of pollution in aquatic ecosystems (Mitchell and Kennedy, 1992; Park *et al.,* 1993). Fish species from contaminated areas initiated studies in the aquatic environment (Murchelano and Wolke, 1991; Mc Mahon, 1994; Moore and Myers, 1994) and evidence is growing that environmental mutagens can reduce the reproductive success of populations (Anderson and Wild, 1998). Different genotoxicity tests and their applications to environmental monitoring and assessment have been variously reported in fish (Hartmann *et al.,* 1999; Gartiser, 2000; Gartiser *et al.*, 2001; White *et al.,* 1998a; White *et al.,* 1998b; Helma *et al*., 1996; Vargas et al., 2001; Hose *et al*., 1998; Stahl, 1991; Mitchelmore and Chipman, 1998b; Mulleschon 1989; Grummt, 2000b). Fish cells retain important traits of fish; for example, poikilothermic behaviour, unique xenobiotic metabolism, and low rate of repair mechanism (Kapour and Nagpure, 2005). DNA repair has been shown to be slower in fishes than mammals (Walton *et al*., 1984; Espina and Wesis, 1995). Therefore, they can be used as sentinel organism for biomonitoring studies (Landolt and Kocan, 1983). Fish have severally been used in several eukaryotic genotoxicity and mutagenicity tests, which include its use in Comet assay (Sumathi *et al*., 2001), DNA repair synthesis (Mullerschon, 1989; Grummt, 2000b), Chromosomal aberration test (Al-Sabti, 1985; Rishi and Grewal, 1995), Micronucleus assay (De Flora *et al*., 1993; Saotome and Hayashi, 2003; Pantaleao *et al*., 2006), and Sister chromatid exchange test (Kligerman et al., 1984; Sahoo *et al*., 1998). Therefore, efforts should be made to utilize assays for detecting genotoxicity caused by aquatic pollutants in fishes at DNA level. This will help in formulating long-term strategies for fish conservation programme besides estimating safe Level of pollutants in water (Kapour and Nagpure, 2005). Recent advancement has been made in the field of ecogenotoxicology (Amanuma *et al*., 2000; Winn *et al*. 2000; McMahon, 1994; Bhaskaran *et al*., 1999; 2000), which use has also been recommended for in genotoxicity testing of new chemical entity (NCE) and pharmaceuticals by the International Conference on Harmonization (ICH) (Jena *et al*., 2001). However, several drawbacks have hindered the effective use of genotoxicity tests in ecogenotoxicology (Wurgler and Ramer, 1992; Anderson and Wild, 1994; Jena *et al*., 2001; OSPAR, 2002). Global efforts should be intensified and harmonized to solve some of these problems such as validating test systems to detect aneuploidy by anticentromere antibody (Nath *et al*., 1995), identification of apoptosis (Abend *et al*., 2000), use of fluorescent *in situ* hybridization (FISH) to visualize translocation of chromosomes (Marzin, 1999; Shimizu *et al*., 2000), unscheduled DNA synthesis (UDS) (Butterworth *et al*., 1987), and cell transformation assay (Martelli *et al*., 2000) in fish. All the foregoing genotoxic screening methods, except apoptosis and unscheduled DNA synthesis (UDS), which have been used in fish (Grummt, 2000b; Singha, 2005), have only been reported in man. Appropriate screening tests should also be validated for investigating consequences of genotoxins, not only on populations, but also on gene pool. These tests will increase both the sensitivity and specificity of existing test protocols (Jena *et al*., 2001).

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