General Consideration

Wildlife toxicological concern can be divided into two broad areas; direct concern for wildlife health and the use of wildlife as indicators of environmental quality. In both areas, it is important to be able to carry out controlled laboratory experiment as well as field studies. In order to identify specific substances that pose potential hazards to amphibians, there is a need to gather data on the toxicity of these contaminants to amphibians in laboratory assays and field experiments as well as their general effects on populations. From this information, an evaluation can be made as to whether amphibians can be employed as useful indicators of environmental quality (Power *et al.*, 1989). Such an attempt needs extensive data of the laboratory and field studies. A review of literature on the amphibian toxicology reveals that in comparison to fishes and mammals very little information exists on amphibians (Power *et al.*, 1989). The present investigation, which includes both the laboratory and field studies, would certainly contribute some information to the requirement of the above objective.

The accumulation of genetic information was a suggested plan of action at the DAPTF workshops, though no major genetic studies have yet been initiated. Certainly, any such information can provide a basis for future studies, which would increase the efficiency of monitoring strategy for amphibian populations (Bogart, 1992).

Because of the larger size and less 2n numbers, amphibian chromosomes have been the subject of many recent investigations. Most of these studies deal with the sex chromosomes (Ohta, 1987; King, 1990; Mahony, 1990; Schmid, 1978, 1980, 1982; Schmid and Almeda, 1988; Schmid and Steinlein 1991; Schmid *et al.*, 1981, 1993)or the effect of temperature on the lampbrush chromosomes (Angelia *et al.*, 1990; N'Da and

Angelia, 1990; N'Da *et al.*, 1990; Rodriguez-Martin *et al.*, 1991). Very few studies are focused on the genotoxicity (Chakrabarti *et al.*, 1984; Banerjee, 1989: Zakidov *et al.*, 1993, Kraskowski *et al.*, 1986; Jaylet *et al.*, 1987). Probably, the technical problems might have hampered the progress of cytogenetic studies on amphibians, especially the *in vivo* mutagenic studies. The fatty droplets surrounding the bone marrow or spleen cells in various species of amphibians often preclude the analysis of chromosome complement (Singh *et al.*, 1974). However, it has been found during the present study that the fat in bone marrow, is not a major problem for the analysis of chromosomes, because a longer fixation can remove the fat content in the metaphase cells (Chapter 1). Another major problem is the seasonal variation in the activity of bone marrow. Generally, amphibians lack an actively hemopoietic bone marrow unlike mammals. Year-round analysis of mitotic activity in the bone marrow of the frog revealed that activity increases from August and reaches its peak between October and January months followed by a sudden decline in February (Chapter 2). Data clearly suggest that the frog *Rana tigerina* can be used as an indicator species for mutagenic assays only during October to January season. During other seasons the mitosis in the bone marrow was found to be negligible. Therefore in the present study, the mutagenic assays on the test animal were confined to the period during October to January.

The results of the mutagenic assays reveal that amphibian chromosomes are prone to aberration with the induction of heavy metal contaminants. It is suggested that metals form covalent and coordinate complexes with the biological macromolecules, which may be the cause of their mutagenic properties leading to metabolic disturbances and genic changes (reviewed by Sharma and Talukder, 1987). Several heavy metal salts used in the present study, especially $CdCl_2$, $HgCl_2$ and $NiCl_2$ were found to have deleterious effect on the chromosomes of the frog (Chapter 3). A comparative assessment of the toxicity of three metallic chloride salts shows that $HgCl_2$ is the most toxic compound. Cadmium chloride was less toxic than $HgCl_2$ while $NiCl_2$ was the least toxic. The types of aberrations observed were almost similar in all the experiments. However, an increase in dicentric chromosome was noticed after treatment with $NiCl_2$. C-mitosis was found occurring in all the treatments though it was predominantly found after the treatment with $HgCl_2$. An increased number of pulverizations were observed when treated with $CdCl_2$. A dose and time dependent

increase in the incidence of chromosome aberration was generally noticeable after the treatments. The mitotic index showed, generally an increasing trend after the treatment with $NiCl_2$. High dose of $HgCl_2$ caused increased mitosis while the treatment with $CdCl_2$ caused only decrease in the mitosis. Thus it appears that though the types of aberration caused by these heavy metals are almost similar, the intensity and the mechanism of action of these compounds widely differ; the mutagenicity of these chemicals on amphibian species may also be different from that of mammals.

Significant number of chromosomal aberrations were induced in the bone marrow of the frog when treated with the effluent water containing high amount of cobalt. However, separate experiments conducted with the cobalt alone indicated that the mutagenicity of the river water was not solely due to the presence of cobalt. Mutagenicity could also be caused by the low pH or amount of other contaminants present in the water or more likely, due to the combination of all these factors (Sharma and Talukder, 1987). Nevertheless, a dose dependent increase in chromosomal aberrations was noted after the treatment with the effluent water, which clearly suggests the clastogenic property of this water on the frog chromosomes.

Lead acetate could produce only a low percentage of aberrations in the frog bone marrow when injected intraperitoneally. Aberrations were increased only when the frogs were treated for 14 days in the lead solution. Since chromatid and acentric fragments were less frequent and complex aberrations such as chromatid exchanges and multiple breaks were absent, Lead acetate could be assumed to exert only a weak clastogenic action on bone marrow cells of frogs. A similar action of this compound was reported in rats (Tachi *et al.*, 1985) and mice (Savic *et al.*, 1986).

The mitotic index was found to be increased in almost all the treatment especially when treated with effluent water and heavy metal salts such as $NiCl_2$ and Lead acetate. Even when treated with other heavy metal salts of mercury and cobalt, the mitotic indices were not found to be decreased as usually reported in case of mammals (Sharma and Talukder, 1987). Significant decrease in mitotic index was recorded only after the treatment with $CdCl_2$.

Apart from contributing to the environmental research, cytogenetic assays for detrimental chemicals may also enhance some data to our understanding of chromosome physiology and drug action (Hsu, 1981). In the present study, it was found that centromere separation and colchiploidy (C-mitosis) were prominent among the aberrations while chromatid gaps and chromosomal breaks were minimum. Chromatid breaks were mostly found near the centromere; break at the middle of a chromatid was not observed in any case. It seems that centromeric heterochromatin region is highly sensitive to breaks in amphibian chromosomes. Studies carried out in *Bufo* species also point to the fact that centromeric heterochromatin is more prone to breaks than the euchromatin in amphibian chromosomes (Banerjee *et al.*, 1989). According to the review of Sharma and Talukder (1987) on the clastogenic effects of the various metals on the bone marrow cells of mammals, the chromatid break is a common type of aberration in mammals. Chromosomes of some mammalian species possess some weaker spots or hot spots, which are more vulnerable to breaks by odd agents like carcinogens or mutagens. It is suspected that the sensitivity of the centromeric heterochromatin to the breaks and separation is due to the special organization of amphibian chromosomes. Structural organization of genome amphibians is different from that of mammals. Schmid and Almeda (1988) have reported that restriction enzymes induce no multiple G- band pattern in amphibian chromosomes, unlike in mammals and birds. The degree of chromatin condensation is also higher in amphibia than in warm-blooded animals (Schmid, 1978). The strong spiralization of the amphibian chromosome may be one reason for the less frequent chromatid gaps. In addition, the DNAs of the cold blooded vertebrates have less DNA in the heavy, dG+dC rich fractions than do the warm-blooded vertebrates(Bernadi *et al.*, 1985). Amphibian DNA does not exhibit the long base composition homogeneous regions present in mammals and birds (Bernadi *et al.*, 1985) These evidences well appreciate the difference between the mammals and amphibians in the structural organization of the genome. However, extreme fragmentations and multiple aberrations were found in the frogs treated with high dose of chemicals for long periods especially with the effluent water. This implies that even the euchromatin regions are susceptible to breaks when the toxic level increases. In general, it could be stated that the centromeric heterochromatin is more prone to breaks and aberrations than euchromatin in amphibia when the chromosomes are subjected to breaks with a chemical mutagen. This difference in clastogenic sensitivity can be attributed to the intrinsic nature of amphibian chromosomes.

The sensitivity of amphibians to organic or inorganic compounds may allow environmental monitoring of these compounds or local changes in the environment using amphibians as indicators once standardized techniques and representative species have been established. On the other hand, the diversity and distribution of amphibians species could also provide a picture on the quality of our environment as environmental pollution has been attributed to the major reason for the declining amphibian population. Second part of this investigation has been concentrated on some field studies carried out in the Narmada valley in Gujarat region. Studies conducted in this area revealed the presence of 13 species of amphibians in the Shoolpaneshwar sanctuary. The species richness in the sanctuary has been compared with other parts of Gujarat. Various physical and biological attributes that affect the species richness in this area were also analyzed. It has been revealed that the species richness in this area depends upon various factors and no single factor can be responsible for enhancing and maintaining the diversity.

Maximum species richness was found in the core area of the sanctuary, where there is good canopy with minimum biotic interference as well as maximum quantity of biomass and leaf litter. Habitat heterogeneity is a factor that enables a large number of ecologically related species to co-occur in a same environment. Rich vegetation of the sanctuary is composed of moist and dry deciduous as well as evergreen trees (Pradeepkumar, 1993). Such trees can provide a variety of habitat resources and can behave actual colonies of organisms. There are enough flowers round the year in the sanctuary, providing food to large number of insects and birds. The maintenance of a high animal species diversity mostly depends on the steady availability of a wide and diverse array of food resources. The sanctuary supports the habitats for a wide variety of insects, a principal prey for amphibians (Radhakrishnan *et al.*, unpublished).

Ultimate determinant of the high species richness in an area is related to the sustained primary productivity of the ecosystem concerned and in their floral diversity on both land and in water. A comparatively high rainfall results in greater plant productivity, which provides an abundance of food to primary consumers, most of which are insects. The sanctuary provides a large number of undisturbed areas characterized by closed canopy that promotes moist soil conditions and vegetation

resulting in substantial amounts of detritus material in both aquatic and terrestrial environment and thereby strengthening a complex and complete nutrient cycle. Well-balanced ecosystems exist in most part of the sanctuary.

The analysis of distribution of amphibians in different agroclimatic regions of the Gujarat State indicates that the South Gujarat region is richest in species diversity, where 17 out of 19 species are present. This can be primarily attributed to the better rainfall and the climatic conditions. Though the Central Gujarat receives higher rainfall than the Saurashtra and having a similar soil conditions, the species richness in the former region is less than the latter. This is probably due to the increased industrialization prevailing in the Central Gujarat. Even the species richness in the South Gujarat area is merely confined to the Dangs and Shoolpaneshwar sanctuary, probably due to the impact of pollution in other parts of this region.

The high species diversity of amphibians in an area certainly depends upon the climatic factors, geographical and geological features, which in turn determines a good primary productivity, habitat heterogeneity and an over all well balanced ecosystems where there is less human intrusion. Nevertheless, the impact of pollution is the most important factor than other biological or physical attributes in determining the species diversity of an area. Moreover, the laboratory studies conducted in the present investigation substantiates this concept. Heavy metals such as Mercury, Cadmium and Nickel were found to have significant clastogenic effect on the frog chromosomes in its sublethal concentrations. Clastogenicity increases when the heavy metals present in the water along with other impurities. These short-term studies imply that the environmentally induced chromosomal aberration could have a deleterious effect on the genome of amphibian species and could be an important factor contributing to the decline of the population of this taxa.

It is a fact that short-term projects cannot form the basis for solving environmental or biological conservation problems. Ecology requires long-term studies, which demands protected areas and long-term funding (Brooks, 1992). Nevertheless, most long-term studies develop originally from short-term studies (Callahan, 1984), which are essential in dealing with long-term problems that require historical data or in testing most hypotheses in ecology.