3. FACTORS AFFECTING TOXICITY

Various characteristics of xenobiotics, their exposures, surrounding medium and organisms affect their toxicity. Characteristics which affect the toxicity of a chemical are considered as modifying factors. The factors pertaining to the chemicals are: (*i*) physico-chemical properties including the functional groups, (*ii*) solubility in water and organic solvents, (*iii*) dose/concentration, (*iv*) ionic characteristics (*v*) translocation and biotransformation, (*vi*) their mode of action, and (*vii*) interaction with other chemicals. Almost all these characteristics are dependent on the structure of compound. The factors related to the exposures are: (*i*) routes of exposure, (*ii*) exposure systems, (*iii*) exposure duration, *etc.*

The factors pertaining to the surrounding medium affecting the toxicity of xenobiotics have been worked out in a greater detail for the aquatic medium. Hence, the effects of physico-chemical characteristics of water on the toxicity of chemicals are more understood. Therefore, the role of these factors in the toxicity of chemicals will be considered in this chapter at length. These factors are: (*i*) water temperature, (*ii*) dissolved oxygen, (*iii*) pH, (*iv*) salinity, (*v*) water hardness, and (*vi*) suspended and dissolved substances.

Besides, dissolved gases, such as CO_2, photoperiod and intensity of light, water movements, binding and chelating action of substances mostly present in natural water also affect the toxicity of chemicals; but there is paucity of data on these aspects. The toxicity modifying factors related to the chemical, the exposure and the surrounding medium may be considered together and termed as *abiotic modifying factors*.

The factors pertaining to the organisms are: (*i*) type of species, (*ii*) sex, (*iii*) age, (*iv*) stage of the life cycle, (*v*) weight and size of individual, (*vi*) health and nutritional status, (*vii*) seasonal physiological state, and (*viii*) acclimation of individuals. The factors related to the organisms may also be termed as *biotic modifying factors*. For the sake of convenience, this chapter will be divided into following four sections:

1. Factors pertaining to chemical,
2. Factors pertaining to exposure,
3. Factors pertaining to surrounding medium, and
4. Factors pertaining to organisms.

1. Factors pertaining to chemical

(*i*) ***Chemical composition*** The physico-chemical characteristics of a compound, such as solubility, vapour pressure, ionization, functional groups, *etc.* are largely governed by the chemical constitution of the compound. All these characteristics greatly affect the toxicological properties of the substance. The nature of chemical reaction is determined by the functional groups of the chemical and toxicity is a response of organisms consequent upon reaction between chemical and certain part of the organism. The polar (hydrophilic) chemicals are not easily soluble in lipids. Therefore, they cannot easily cross the membranous barriers and thus cannot easily reach the target sites for appropriate action. However, non-polar or lipophilic substances are highly soluble in lipids and other organic solvents. They can readily penetrate the lipoprotein

layers of membranes, hence they readily produce their potential effects. It can thus be inferred that the toxicity of a chemical is largely dependent on the chemical structure, type of functional groups, ionic characteristics and lipid solubility.

(*ii*) ***Dose or concentration of chemical*** A chemical induces toxic effects on account of its interaction with appropriate receptors. The effect is directly related to concentration of the chemical at the target site and concentration at the target site is often directly proportional to dose/concentration of the chemical exposed. The lower doses of chemical cause less effects whereas higher doses may cause pronounced effects. Thus, toxicity of chemical is directly proportional to its dose/concentration.

(*iii*) ***Translocation of toxicant*** There are two types of toxicants:(*a*) those producing local effects, and (*b*) those producing systemic effects. In case of the latter group, effective translocation is a key factor. Unless the toxicants are readily translocated to the specific sites, they cannot produce adverse effects. During the course of translocation, some of the toxicants interact with certain macromolecules present in the body of organisms and are then stored in certain relatively inactive tissues, *i.e.* ***storage depots.*** The inefficient translocation and storage of toxicants in inactive forms reduces their toxicity, while their effective translocation to the target in active forms enhances their toxicity.

(*iv*) ***Biotransformation of toxicants*** Certain chemicals are normally inactive. During translocation such chemicals are biocatalytically converted into active form in the body of organisms with the help of certain enzymes. Consequently, the toxicity of that particular chemical is increased. For instance, organophosphorodithionates and organophosphorodithioates (having P=S group) are relatively poor inhibitors of AChE. Therefore, in the body of organisms, they are first converted to their P=O forms, which are potent AChE inhibitors, hence highly toxic. Thus, biotransformation of xenobiotics leading to their activation, enhances the toxicity of the compound. However, in certain other cases active xenobiotics are converted to their inactive forms during translocation and these forms are often stored in the nontarget or relatively inactive tissues. If the biotransformation leads to conversion of lipophilic form to the hydrophilic form, the excretion of xenobiotics may be enhanced. Thus, toxicant is not available at the target sites to produce adverse effects. This latter type of biotransformation decreases the toxicity of xenobiotics.

(*v*) ***Chemical interactions*** In nature often various chemicals may be present and the organisms are not exposed to only one chemical, but to a variety of chemicals. These chemicals interact with each other and such chemical interactions may have great toxicological significance. However, in the laboratories, the organisms may be simultaneously or consecutively exposed to two chemicals. These chemicals interact and affect the toxicity of each other. These interactions may cause three types of toxic effects.

(*i*) The combined effect of two chemicals may be equal to the sum of the effect of each chemical when given alone. This type of interaction is considered as ***additive***; for example, combinations of various organophosphorus pesticides and their effects on AChE activity.

(*ii*) The combined effects of two chemicals to the organisms exposed may be greater than the sum and this type of interaction is designated as ***synergistic***; for example, effects of carbon tetrachloride and ethanol on liver; and asbestos exposure and cigarette smoking on the lungs.

(*iii*) The combined effect of two chemicals to the organisms exposed may be less than the sum and this type of interaction is termed as a***ntagonistic***; for example, chelation of heavy metals by dimercaprol.

It may thus be inferred that interaction of one chemical with another may:

(*i*) have no effect on their toxicity, or

(*ii*) increase their toxicity, or

(i) decrease their toxicity.

2. Factors pertaining to exposure

Toxicity of chemicals is greatly affected by various factors pertaining to their exposures to the organisms. Some of the important factors related to exposures are: (*i*) exposure routes, (*ii*) exposure duration, and (*iii*) exposure systems.

(*i*) ***Exposure routes*** The toxicant gain access to the body of organisms by dermal, oral and inhalation exposures or by intraperitoneal, intramuscular, subcutaneous and intravenous injections. The routes of exposure largely affect the toxicity of chemicals. A chemical produces more rapid and greatest effect when given by intravenous route, because through this route the chemical directly reaches in active form to the specific site and thus produces greatest effects. The other exposure routes in descending order of toxicity are: inhalation> intraperitoneal>subcutaneous>intramuscular>oral>topical.

(*ii*) ***Exposure duration*** LC_{50} or LD_{50} values of toxicants decrease with increase in duration of exposure. The values evaluated for long-term exposures are much less than those determined for short-term exposures. The exposure to the same dose or concentration of a toxicant for different periods has been reported to induce different grades of effects. The exposure for short duration has less effect in comparison to long-term exposures. This simply indicates that the toxicity of substances increases with increase in exposure period.

(*iii*) ***Exposure systems*** A variety of exposure systems may be used for the exposure of toxicants. For instance, in aquatic medium, various exposure systems are used for the exposure of toxicants to organisms, such as:

(*a*) *Static system* Where toxicant is mixed in the water and the organisms are exposed to it in still water.

(*b*) *Recirculatory system* Where the toxicant solution is recirculated through certain pumps.

(*c*) *Renewal system* Where the toxicant solution is renewed after certain interval.

(*d*) *Flow through system* : Where the toxicant solution flows into and out of test chamber intermittently or continuously.

These various types of exposure systems do affect the toxicity of chemicals to the organisms to a great deal.

3. Factors pertaining to the surrounding medium

The factors of surrounding medium affecting the toxicity of chemicals have been extensively worked out for the aquatic medium. The factors related to the medium are physico-

chemical characteristics of the water affecting the toxicity of chemicals. Hence, those parameters are considered for this section.

(*i*) ***Water temperature*** The water temperature is expected to greatly affect the toxicity of xenobiotics. The increased water temperature increases the solubility of many substances, affects the chemical form of some and governs the amounts of dissolved oxygen in water. Studies by various investigators suggest that there is no single pattern of the effects of temperature on the toxicity of chemicals to aquatic organisms. Temperature change in a particular direction may increase or decrease or cause no effect on the toxicity of chemicals, depending on the chemical, the species, the response and the particular procedure. Changes in water temperature may not have much effects on chronic thresholds of chemicals, which are ultimately of great importance to aquatic organisms.

Several pesticides have been reported to be more toxic at higher temperatures while some others show stronger lethal action at low temperatures. Sanders and Cope (1966) evaluated the toxicity of 22 chemicals in relation to different temperatures against cladocerans and found that low temperature causes stronger lethal action. The findings were particularly effective in case of DDT toxicity. DDT was three times more toxic at 10ºC than at 27ºC. Contrary to these findings, Omkar (1982-83) during the course of his toxicity studies of pesticides to fresh water prawns noticed that increase in temperature of the medium (*e.g.* water) increases the toxicity of pesticides to the organisms exposed. Similar findings were also reported in case of insect larvae, fish species, mollusks, *etc*. in relation to pesticides and several other chemicals.

(*ii*) ***Dissolved Oxygen*** Freshwater can dissolve 14.6 mg/1 level of oxygen at 0ºC, which gradually decreases to 9.1 mg/1 with increase in temperature upto 20ºC and reaches to the level 7.5 mg/1 at 30ºC. It is, therefore, obvious that increase in temperature decreases the dissolved oxygen content of the water. Oxygen is essentially required for respiration by the organisms. Any physiological change affecting the rate of respiratory flow of aquatic organisms may affect the concentration of the toxicant at the gill surface and thus the toxicity of the chemical. Thus, it might be expected that reduction in dissolved oxygen content of water imposes stress on the aquatic organisms, which may greatly increase the toxicity of a chemical in water. Infact, decrease in dissolved oxygen content of water increases the toxicity of chemicals to aquatic organisms. But, it is not a major modifying factor, because most of the findings show a change in the toxicity of chemicals by only a factor of two or less for tests conducted at low and high levels of dissolved oxygen.

(*iii*) ***pH*** pH may have greater effects on the toxicity of those chemicals that ionize under the influence of pH. Usually undissociated forms of chemicals are more toxic to organisms, because they easily penetrate the cell membranes. For instance, most of the pesticides do not easily dissociate and owing to their lipophilic nature, they easily penetrate through the lipoprotein bimolecular layer of cell membranes to induce the toxicity. Thus, pH does not seem to significantly affect the toxicity of such chemicals.

The toxicity of ammonia is known to be greatly affected by pH of the water. Therefore, the effect of pH on the toxicity of ammonia may be taken as an example. Unionized form of ammonia (NH_3) is highly toxic to fish and the toxic range is quite low (0.2-0.7 mg/1) for salmonids. In contrast, ionized form of ammonia (NH_4^+) has very little or no toxicity. A rise of one pH unit within the usual middle ranges increases the proportion of ammonia (NH_3) by about six folds, hence six folds increase in the toxicity of substance.

Another example of the effect of pH on toxicity of chemicals is cyanide toxicity. Appreciable proportion of ionic cyanide (CN^-) occurs at pH 8.5, whereas the molecular form (HCN) predominates at acidic and middle pH values. Undissociated form of cyanide is twice as toxic as ionic form. Hence, changes in water pH may greatly affect the toxicity of cyanide.

The most of the metallic chemicals behave in a different way. pH has pronounced effect on the toxicity of metals, because they ionize under the influence of pH. The ionic forms of metals are more toxic to organisms than undissociated or molecular form. Therefore, changes in pH in either direction may increase or decrease the ionic proportion and this in turn may increase or decrease the toxicity of metallic compounds.

(*iv*) ***Salinity*** The greatest differences in chemical characteristics of fresh water and seawater may be expected to enormously affect the toxicity of chemicals. But, these differences do not significantly alter the toxicity of chemicals. The tolerance between the close relatives of fresh water and marine organisms are almost equal, if both are tested in their own environments. Thus, it may be inferred that in general salinity does not significantly affect the toxicity of xenobiotics. However, a particular species may be more affected with changes in water salinity.

Generally, euryhaline organisms are more resistant in about one third seawater (*i.e.* water containing 30-40% salinity), in salinity close to their isosmotic level. However, appreciable decrease in salinity of water often renders the marine animals less tolerant. Therefore, it may be concluded that the toxicity of xenobiotics may increase with appreciable decrease in the salinity of the surrounding medium.

Herbert and his associates (1964 and 1965) investigated the effect of salinity on the toxicity of various chemical pollutants to rainbow trout and Atlantic salmon. They inferred that the toxicity of zinc and ammonium chloride decreases with increase in salinity (*i.e.* from fresh water to water containing 30-40% salinity), level of their isosmotic concentration and thereafter the toxicity further increases. A similar relationship has been established after the study of naphthalene toxicity to euryhaline mummichog. The less mortalities of mummichogs in low salinities than in high salinities have been ascribed to osmoregulatory dysfunction and increased uptake of naphthalene.

(*v*) ***Water hardness*** The total hardness of water has little effect on the toxicity of most of the chemicals except for metals. The toxicity of ammonia, phenols, surfactants and pesticides has been reported to be affected the least by the hardness of water.

LAS, a surfactant, has been reported to be 1.5 times as toxic to bluegills in hard water as in soft water. In contrast, a comparison of toxicity of 14 petrochemicals in hard and soft water revealed significant differences for only four of them and those were generally two times more toxic to fathead minnows in soft water than in hard water (Pickering and Henderson, 1966). Similarly, of the four hydrazines tested, only two of them showed differences in toxicity. Hydrazine was 6 times more toxic in soft water, while dimethyl hydrazine was 2.5 times more toxic to guppies in hard water. Out of the various pesticides tested, only two carbamates showed three-fold increase in toxicity with decrease in water hardness (Macek *et al.*, 1977).

Metal toxicity is greatly affected by the hardness of water. Many heavy-metals are more toxic to fish and other aquatic organisms in very soft water in contrast to hard water. The increase in toxicity of metals with respect to decrease in hardness of water has been attributed to changes in gill permeability of fish caused by their calcium content.

(*vi*) *Suspended and dissolved matter* Natural water often contains suspended and dissolved matter including organic ligands and chelators. They may partly detoxify some of the xenobiotic chemicals as a result of sorption or binding. The metals are chief examples that may be detoxified by this mechanism.

There is very scant literature on the effect of sorption and binding of other non-metallic xenobiotics with suspended and dissolved matters. The addition of clay suspension affects the toxicity of an insecticide, endrin, to fathead minnows. In contract, the addition of soil suspension to endrin solution moderates its toxicity.

The addition of clay suspension at levels normally present in nature did not affect the toxicity of the surfactant, LAS. Thus, it may be concluded that the toxicity of metals is often greatly decreased by the suspended and dissolved matters present in water because of sorption and binding while other xenobiotics are much less affected.

4. Factors pertaining to organisms

Toxicity of chemicals is evaluated against a particular organism or a group of organisms. Therefore, the factors related to the organisms are very important in the study of toxicity of xenobiotics. The important modifying factors pertaining to organisms are outlined below:

(*i*) ***Test species*** The toxicity of xenobiotics greatly varies with variation in test organisms, as the tolerance to chemicals differs in different groups of organisms. Among the organisms of same group, the toxicity of chemical varies with variation in species of the organisms. Even in different individuals of same species, the toxicity of chemicals varies because of variation in susceptibility owing to certain genetic factors. Thus, certain individuals of a species may be susceptible to a chemical whereas the other may be resistant to the same chemical. Owing to these variations, it is often suggested that to have a clear picture of the toxicity of a chemical, it must be tested against one plant (may be an alga), one or two invertebrates and at least one vertebrate. Thus, in aquatic medium a chemical may be tested against an algal species, an aquatic insect or crustacean and at least one fish. In general the invertebrates are sensitive to toxicants than the vertebrates.

The toxicity of a chemical may vary among various fish species. Spear and Pierce (1979) reported that salmonids and minnows are about 15 times more susceptible to copper than that of sunfish. Extreme examples of differences between various species have been reported in the toxicity of organophosphorus pesticides. Pickering *et al.* (1962) found 6 to 900 fold variations in the sensitivity to these pesticides between sensitive (bluegills and guppies) and tolerant species (fathead minnows and goldfish).

(*ii*) *Sex* The toxicity of chemicals differs with respect to sexes, because the males and females differ in their responses due to hormonal and metabolic differences. Males in some species biotransform compounds more rapidly than females, although this is not true for all species. For instance, aldrin (an organochlorine pesticide) is much more toxic to male rats than to female rats, because aldrin is biocatalytically converted to epoxide form, dieldrin, by the males and this biotransformation product is more toxic than the parent compound. Contrary to this, parathion is bioconverted to paraoxon at a faster rate by the female rat than the male, which is more toxic than the parent compound. Therefore, female rat is more susceptible to parathion than the male.

Besides sex-wise metabolic differences, differences in routes of excretion also occur, which may cause differences in susceptibility. Generally, biliary excretion of glucuronide conjugate is favoured in male while urinary excretion predominates in female. For example,

dinitrotoluene induced tumour occurs predominantly in males due to differences in route of excretion. Chloroform induced kidney damage in mice may also be taken as an example of difference in susceptibility having metabolic and hormonal basis. The male mice are more susceptible to chloroform induced damage and it is believed that male sex hormone, testosterone, affects the microsomal enzyme mediated biotransformation of chloroform.

(*iii*) *Age* Generally, the young animals are more susceptible to xenobiotics. For a majority of chemicals, the youngs are 1.5 to 10 times more susceptible than the adults. The main reasons for the susceptibility of young ones may be less resistance and lack of biotransformation enzyme systems. It has already been reported that the newly born individuals do not posses the enzyme systems catalyzing the biotransformation reactions. These enzyme systems develop gradually, reach at peak at a certain stage, thereafter tend to decline. In accordance with biotransformation reactions involving detoxication of xenobiotics, the susceptibility of organisms may also change. But, it is not necessary that all chemicals are more toxic to the young ones. Certain CNS stimulants including DDT are less toxic to young ones.

(*iv*) *Life-stage* The toxicity of chemicals varies with different stages of the life-cycle. Generally, the early life-stages or immature stages are more susceptible to toxicant exposures than the late stages or mature individuals. The fries and fingerlings of fishes are most sensitive stages. Omkar (1980-81, unpublished data) has also found juveniles of freshwater prawns to be more susceptible to pesticide exposures than those of adult individuals.

In certain organisms or a group of organisms, a particular stage of the life-cycle may be particularly susceptible to toxicants and exposure of toxicants at this stage appreciably affects the results. For instance, the time of moulting is particularly susceptible in case of aquatic arthropods. Omkar (1980-82, unpublished data) has observed moulting in prawns during pesticide exposures and these moulted individuals died later on while others of the same batch survived till the end of the tests.

(*v*) *Size* The toxicity of chemicals is also affected by the size of the organisms. Often larger sized individuals are more resistant to toxicants and this has been found true in case of certain fishes. Hearth and Sprague (1978) reported that copper tolerance to rainbow trout gradually increases with increase in size of the fish. While, Anderson and Spear (1980) on the basis of their studies on copper toxicity to fish had concluded that lethal tolerance to copper may or may not vary with the fish size.

Sanders and Cope (1968) have tested some pesticides to three species of stonefly naiads and have concluded smaller species to be more susceptible to pesticides than the larger species. Omkar (1980, unpublished data) has also tested various pesticides against a freshwater prawn and experienced smaller individuals to be more susceptible to those pesticides in comparison to larger individuals of the same species.

(*vi*) *Health and nutrition* The toxicity of chemicals to organisms is affected by the health and nutritional status of organisms. Generally, the healthy individuals are more tolerant to toxicants than diseased ones. The diseased and parasitized individuals have been reported to be more sensitive to various toxicants than the normal ones. For example, unhealthy fishes have been reported to be more susceptible to sodium chloride and an organophosphate pesticide, guthion.

The toxicity of chemicals is also affected by the nutritional status of the organisms. Sosnowki *et al.* (1979) have reported wild populations of marine copepods to be more resistant to copper in the presence of sufficient food.

Diet particularly affects the susceptibility of organisms to pesticides. Supply of protein-rich diet to rainbow trout improves its tolerance to lethal levels of an organochlorine pesticide, chlordane, by six folds. The fish exposed to toxaphene use vitamin C in the detoxication of toxicant. Therefore, increase of vitamin C in the diet enables the organisms to detoxify the chemical. Verma *et al.* (1982) have also reported that the supplementation of diet with vitamin C decreases the toxicity of pesticides to fish. However, excess or deficiency of vitamin C does not change the symptoms of lead toxicity (Hodson *et al.*, 1980).

(v) Acclimation The animals acclimated to sub lethal levels of a toxicant may become more tolerant or more weakened, depending upon the mode of action of toxicant and the types of detoxifying mechanism of the animals. For instance, acclimation of trout to sub lethal (0.22 mg/1) level of arsenic for three weeks, increased the threshold of LC_{50} by a factor of 1.5. While acclimation to one-third of the lethal level of cyanide to fish rendered them to become more sensitive by a factor of one-third in the first week. The tolerance improved to the original level by the end of three weeks.