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# Levels of Toxicity Screening of Environmental Chemicals Using Aquatic Invertebrates - A Review

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Sajal Ray

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## Abstract

Effective screening of the toxicity of chemicals using living organisms has been considered as a major issue of environmental biomonitoring. The principle of toxicity screening involves the quantitation of toxin-induced shift of biological response or tissue morphology of test species both *in vivo* and *in vitro*. Most of the toxin appears to function as biological response modifiers at a defined concentration and span of exposure. In recent years, invertebrates have been gaining a special scientific attention for being utilized as suitable model for toxicity screening. Invertebrates like crab, mollusks, sponge, and earthworm have already been established as model organisms for toxicity screening and analyses. A number of environmental toxins like arsenic, pyrethroid, pesticides, heavy metals, and washing soda can be screened for their toxicities using invertebrate species. Cellular and subcellular parameters like blood cell density, lysosomal membrane stability, cellular damage, apoptosis, micronucleation, and cytotoxic response of invertebrates had been established as biomarkers of environmental toxicity. Toxin-induced histopathological and behavioral shift had been suggested as effective parameters of toxicity screening in model invertebrates. However, reactivity and responses of invertebrates toward xenobiotics are often recorded to be species specific and related to the chemistry of the toxin. Current article reviews different levels of toxicity screening using invertebrate as test model.

**Keywords:** Sponge, mollusks, crab, arsenic, washing soda, pesticides, hemocytes

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## 1. Introduction

Current global environment is characterized by the presence of diverse chemical compounds of inorganic and organic in nature [1]. Multiple human activities have been identified as the sources of origin of these environmental xenobiotics. Various industrial processes and agricultural activities result in the rapid and precarious contamination of the terrestrial and

aquatic ecosystems. Environmental toxins after generation from sources of origin enter the specific ecological compartments following the nonspecific and specific patterns of dispersion [2, 3]. Environmental dispersion of xenobiotics largely depend on their physicochemical characteristics which largely influence the distribution and fate of the toxic compounds related to half life, polarity, environmental and cellular degradability and toxicokinetics [4]. Upon entering the cellular environment, many of these environmental chemicals undergo enzyme guided xenometabolic transformation chemically termed as biotransformation. Xenometabolism of environmental chemicals may exhibit dual modes of transformation i.e. bioactivation and bioinactivation. Both bioactivated and bioinactivated environmental metabolites are the enzymatic products of biochemical degradation. Principal objective of xenometabolism involves transformation of nonpolar chemical compounds into a product with higher polarity for facilitation of urinary excretion. However, classification of environmental toxins distributed in the current global environment had never been an easy task for the environmental chemists. Rapid and uninterrupted contaminations of the global ecosystem by newer generations of chemical compounds have been identified as a serious challenge and require effective screening of their toxicity in suitable animal models. Major classes of environmental toxins that have been characterized by the chemists and biologists include acids, alkalis, pesticides [1], nuclear fall outs, diverse organic compounds etc. With the rapid shift in the overall physicochemical characteristics of global environment, a continuous search for suitable biological systems for toxicity screening of xenobiotics is being carried out by the toxicologists employing invertebrate species. Scientific information available in the recent years indicates the suitability of invertebrates as “test organism” for toxic screening of environmental chemicals (Figure 1).

## 2. Environmental chemicals

Current global environment is characterized by the presence of diverse chemical compounds of inorganic and organic in nature [1]. Multiple human activities have been identified as the sources of origin of these environmental xenobiotics. Various industrial processes and agricultural activities result in the rapid and precarious contamination of the terrestrial and aquatic ecosystems. Environmental toxins after generation from sources of origin enter the specific ecological compartments following the nonspecific and specific patterns of dispersion [2, 3]. Environmental dispersion of xenobiotics largely depend on their physicochemical characteristics, which largely influence the distribution and fate of the toxic compounds related to half life, polarity, environmental, and cellular degradability and toxicokinetics [4]. Upon entering the cellular environment, many of these environmental chemicals undergo enzyme-guided xenometabolic transformation chemically termed as biotransformation. The xenometabolism of environmental chemicals may exhibit dual modes of transformation, i.e., bioactivation and bioinactivation. Both bioactivated and bioinactivated environmental metabolites are the enzymatic products of biochemical degradation. The principal objective of xenometabolism involves the transformation of nonpolar chemical compounds into a product with higher polarity for facilitation of urinary excretion. However, the classification of

environmental toxins distributed in the current global environment had never been an easy task for the environmental chemists. Rapid and uninterrupted contaminations of the global ecosystem by newer generations of chemical compounds have been identified as a serious challenge and require effective screening of their toxicity in suitable animal models. Major classes of environmental toxins that have been characterized by the chemists and biologists include acids, alkalis, pesticides [1], nuclear fall outs, diverse organic compounds, etc. With the rapid shift in the overall physicochemical characteristics of global environment, a continuous search for suitable biological systems for toxicity screening of xenobiotics is being carried out by the toxicologists employing invertebrate species. Scientific information available in the recent years indicates the suitability of invertebrates as “test organism” for toxic screening of environmental chemicals (Figure 1).

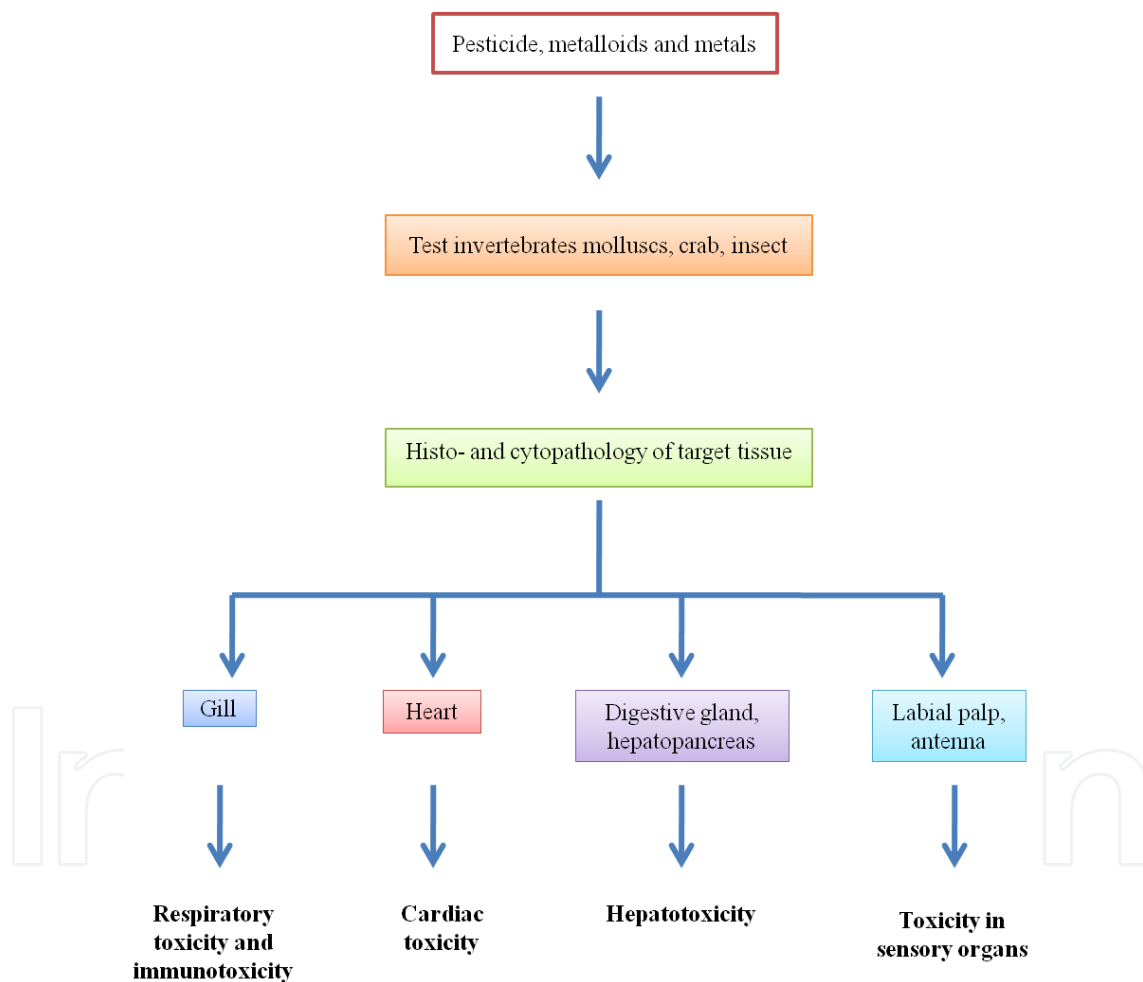


Figure 1. Cellular and organ pathology as major screening parameters of toxins.

## 2.1. Invertebrates and toxicity screening

The anatomical variation of body cavity or coelom greatly influences the physiological and adaptational attributes of invertebrates. Invertebrates without coelomic cavity are termed as

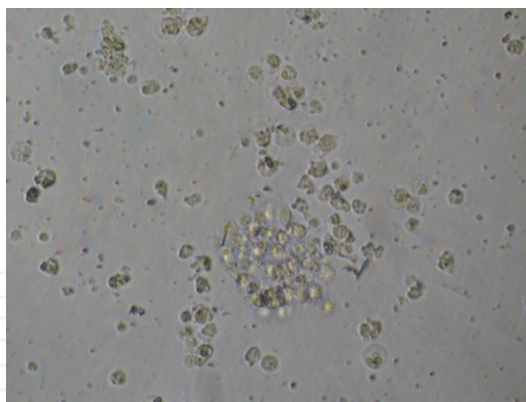
acoelomate, whereas the species with a true coelom is termed as eucoelomate. Pseudocoelomate is a characteristic intermediate group without a true coelom. Invertebrates with open mode of circulatory system evolved blood cells termed as hemocytes and coelomocytes. Circulating blood cells of molluscan hemocoel is known as hemocytes, whereas coelomocytes (Figure 2) are the coelomic cells recorded in the annelids. Recent scientific reports indicate the efficacy of hemocytes and coelomocytes of invertebrates as ideal tools for screening the toxicity of environmental chemicals of known or less known toxicity. Many of these screening methodologies involved both *in vitro* and *in vivo* modes of laboratory testing.



**Figure 2.** Morphofunctional attributes of earthworm coelomocyte reflects the efficacy of soil invertebrates to act as model organisms to screen the toxicity of agrochemicals.

### 2.1.1. Morphological aberrations of invertebrate blood cells as a measure of the toxicity of environmental chemicals

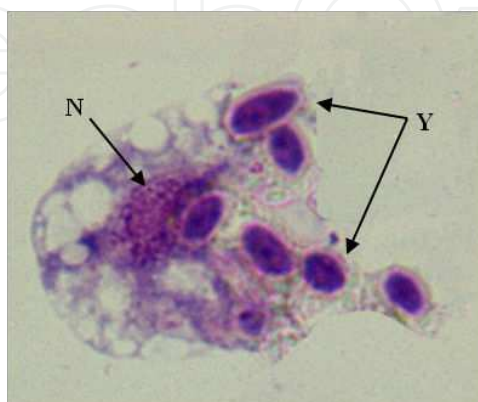
Hemocytes or coelomocytes are the main types of circulatory cells of invertebrates. They perform diverse types of physiological functions and include transport and carriage of gases, nutrients and bioactive substances, nonself recognition, and deactivation of environmental pathogens and toxins. Discrete subpopulations of invertebrate blood cells act as immunocytes and are involved in elicitation of immunological reactivity against environmental toxins and pathogens. Toxins with diverse chemical identities are capable of generating varied degrees of morphological damage or alteration of blood cells of invertebrates [2, 5, 6, 7]. The microscopic examination of morphological aberrations of blood cells thus serves as a unique tool for the testing of the toxicity of environmental chemicals both *in vivo* and *in vitro*. The treatment of the test organisms with measured quantity of chemical toxins *in vivo* provides satisfactory result in experimental toxicology for toxicity testing [2]. The testing of toxicity employing short- or long-term cell or tissue culture technology (Figure 3) occasionally provides an opportunity for the toxicologists in screening the toxicity of xenobiotics. However, invertebrate cell and tissue culture technology appears to be a challenging domain due to the lack of the scientific information and species-specific cellular response in artificial culture media. Hemocytes of aquatic mollusks upon treatment with xenobiotics yield multiple morphological aberrations like vacuolation, membrane disintegration, cellular disruption, and shift in size and shape [2].



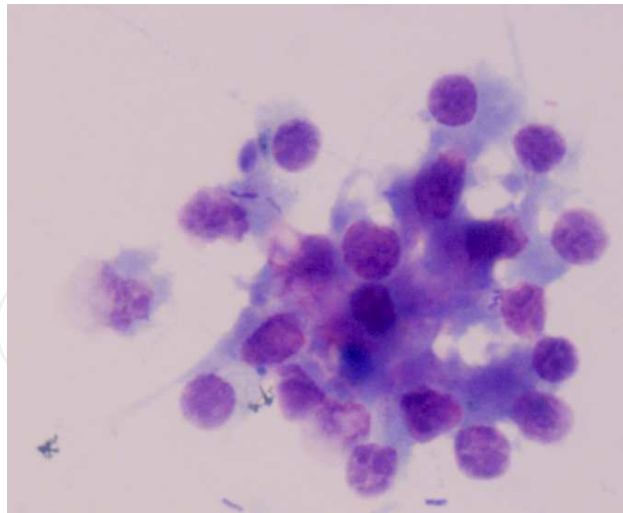
**Figure 3.** Molluscan hemocyte culture is an effective technology of toxicity screening *in vitro*.

### 2.1.2. Functional parameters

Defined functional parameters of hemocytes, coelomocytes, or other body cells may also serve as a tool for screening the toxicity of chemical compounds. Studies of the functional attributes of invertebrate cells require advanced level of bioinstrumentation. Some of the established functional parameters of invertebrate cells include phagocytic response, aggregation response, cytotoxicity, cell doubling time, lysosomal membrane fragility, etc. Phagocytosis (Figure 4) is established as an innate immunological response reported in all major invertebrate Phyla [8, 9]. It is characterized by sequential-like recognition, chemotaxis, contact, and internalization followed by the degradation of the target in the cell's interior. The degree of phagocytic response can be quantitated by suitable cellular index. The toxicity of multiple environmental chemical can suitably be estimated by determining the phagocytic index of invertebrate immunocytes against ideal control. Cellular aggregation (Figure 5) is also recognized as a metabolic behavior of cells against chemicals or foreign particulates of toxic nature. Various molluscan test species exhibit the aggregation response of hemocytes upon the exposure of environmental pathogens and toxins [10].



**Figure 4.** Phagocytic response of sponge cells is an established tool of toxicity screening. N—sponge cell nucleus, Y—yeast particle.



**Figure 5.** Aggregation response of molluscan hemocytes is often modulated by chemical toxins.

### 2.1.3. Dynamics of blood cell density as a measure of toxic response

Blood cell homeostasis of invertebrates is a function of multiple cellular processes. These include rate of hematopoiesis, mitosis, cell migration, and aggregation tendency of hemocytes or coelomocytes. The maintenance of steady state of total count of blood cell depends on the overall health and immunological status [11] of the model organism. The morphological variation of blood cells is not uncommon in the phylogeny. Therefore, differential cell count may also act as a suitable parameter of toxicity screening. However, a discrete scheme of the morphological classification of hemocyte and coelomocyte appears to be absent in many invertebrates [8]. In many cases, the lack of proper nomenclature of hemocyte subpopulation creates a scientific problem in the identification of cells needed for toxicity screening. However, in invertebrates, hemocyte populations like blast cells had been identified as a suitable candidate for the testing of toxicity both *in vivo* and *in vitro* at cellular level.

### 2.1.4. Nuclear aberration and lysosomal membrane fragility of hemocytes and sponge cells

The natural habitats of aquatic invertebrates are often contaminated with diverse toxins of known or less known chemistry. Thus, the hemocytes are being continuously exposed to environmental toxins of varied concentrations. Such a situation often leads to onset of genotoxicity in many of the species belonging to Crustacea and Mollusca. Hemocytes, upon exposure to environmental toxins, may present multiple nuclear aberrations like micronucleation, binucleation (Figure 6), or trinucleation; karyolysis; chromatin condensation; pycnosis; etc. The degree of toxin exposure is often correlated with the magnitude or frequency of nuclear aberrations *in vivo* [5]. Environmental toxins like arsenic, pyrethroid, and alkaline chemical compounds may generate such types of nuclear anomalies in invertebrate. Following this principle, scientists proposed nuclear aberration as a suitable tool for the testing of the toxicity of environmental chemicals. The toxin-induced fragility of lysosomal membrane of hemocytes is in report [2, 7]. Toxin exposure often leads to damage of lysosomal membrane

leading to the release of hydrolases in the cytoplasm. This may result in impairment in the structural profile of cells and tissues. The degree of lysosomal membrane fragility can be quantitated by neutral red retention assay [2, 5, 7] in invertebrates. The lysosomal membrane stability of molluscan hemocytes and sponge cells has been claimed as biomarkers of environmental toxicity (Figure 7).



**Figure 6.** Pesticide-induced binucleation in molluscan hemocytes is a marker of screening genotoxicity.



**Figure 7.** Lysosomal membrane stability of cells is an established parameter of screening ecotoxicity in sponge [7]. Arrows indicate the diffused neutral red probe in cytoplasm of sponge cell.

### 2.1.5. Target enzymes

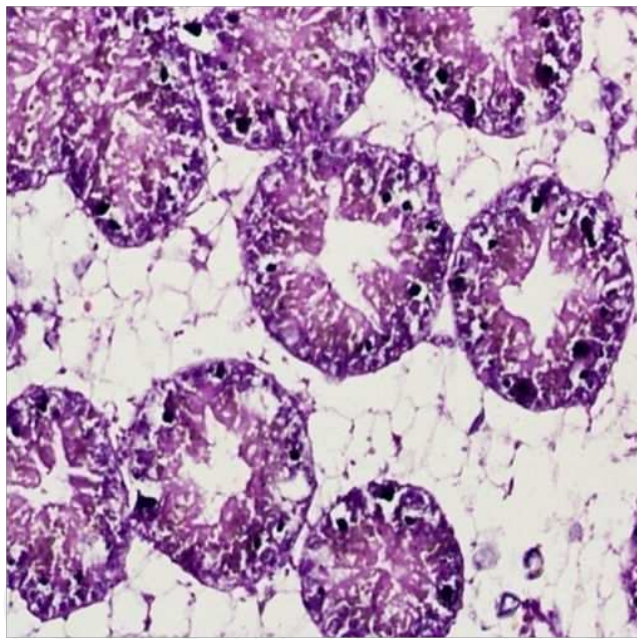
The activity of enzymes as biocatalysts in invertebrate tissues or cells may be considered as effective parameters of assessment of the toxicity of chemicals. Target enzymes of toxins are reported to be located in the cellular or extracellular compartments. Therefore, prior to a biochemical estimation of the activity of these enzymes, physicochemical characterization, and lysate preparation methodology appear to be an important step for the toxicologists. Principal enzymes that are considered and established for toxicity screening include acetylcholinesterase [12], glutamate oxaloacetate transaminase, glutamic pyruvic transaminase [13], ATPase,



phosphatases [13, 7], etc. The activity of acetylcholinesterase is established as an effective parameter of examining the neurotoxicity of many pesticides and allied compounds. The activity of enzymes like glutamate oxaloacetate transaminase, glutamic pyruvic transaminase, ATPase, and phosphatase appeared to be effective and sensitive toward the exposure of many metabolic toxins.

#### 2.1.6. Histopathology

Selected organs or tissues of aquatic invertebrates undergo histological alterations under chronic, subchronic, or acute toxin exposure. Pathological changes of target tissues often provide an excellent scope of assessment of the nature and exposure of chemical toxins (Figure 8). Histopathological analyses had been established as a useful method of toxicity screening in both invertebrates and vertebrates. Histopathology also provides an early signal of pathogenesis and environmental toxicity of diverse toxins of known or unknown chemistry in invertebrates. The magnitude of toxicity by xenobiotics depends on chemical characteristics, route of entry, dosage, span of exposure, and toxicokinetics.



**Figure 8.** Histopathological analyses of digestive gland of marine mollusk, *Telescopium telescopium*, is an effective screening parameter of aquatic diesel toxicity.

Many aquatic invertebrates respire through gill. Gills are highly vascularized membranous structures involved in important physiological process like gaseous exchange with environment, filter feeding, and immunosurveillance [13]. Gills, being relatively exposed to the external environment, interact intimately with the toxic compounds dissolved or suspended in water. This characteristic feature permits gills to act as an ideal organ for histopathological analyses under toxin exposure. In many species belonging to Mollusca and Crustacea, the histopathology of gill had been reported as a suitable procedure of screening the toxicity of

chemical pollutants. Toxin-induced histopathological damage of gill is an indicator in the impairment of the associated functional status of the test organisms. The exposure of the gills of the aquatic bivalves and gastropods to environmental arsenic [13], detergent, pyrethroids, and azadirachtin-based pesticides often lead to the appearance of the hyperchromatic anaplastic cells, clogging of water channels, and lamellar membrane disruption in varied degree.

The exposure of toxins like arsenic and cypermethrin yielded substantial histopathological changes in the heart of the aquatic mollusks [12]. Pathological changes in the auricle and ventricle were prominent under the acute or semiacute treatment of toxins. The unrestricted exposure of test organisms to toxin may thus lead to onset of cardiac toxicity in invertebrates. Histopathological analyses in association of functional assessment of target organs provide an excellent premise of screening the toxicity of xenobiotics. Apart from vital organs like gill and heart, histopathological examination of organs like labial palp [14] of aquatic mollusks had been suggested as an effective procedure of the toxicity screening of pesticides.

#### 2.1.7. Behavioral attribute as a measure of toxicity screening

Behavior in general is considered as a manifestation of physiological performance of organism in both natural and stressed conditions. Invertebrate ethology is relatively a less studied area with limited scientific information. However, behavioral response exhibited by a test species under toxic exposure has been indicated as a method of screening environmental toxicity [14]. Significant deviation in the normal ethogram due to toxin exposure may thus be considered as an effective method of assessment of toxin-induced stress in the test invertebrate.

In mollusks, salient behaviors like relative mobility, aggregation, and mucus release had been established as screening parameters of toxicities of azadirachtin, a neem-based biopesticide. The relative mobility of an organism is often associated with many biological functions like food gathering, mate approach, predatory escape response, etc. [15], in invertebrates. The exposure of toxin adversely affects these parameters which interfere with the functional performance of them and their natural habitat. "Grouping" or aggregation and mucus secretion response had been examined in bivalve and gastropods [16]. Screening of the behavioral toxicity of chemical compounds employing aquatic invertebrates thus appears to be a novel methodology in applied toxicology.

In recent years, invertebrates in general have been gaining a special scientific attraction as model organisms for screening environmental toxicity. Invertebrates occupy diverse habitats with multiple physicochemical characteristics. Their response to a particular ecosystem often appears to be highly specific. Considering the diverse range of global aquatic environment an estuarine invertebrate may appear to be unsuitable for bioresponse assay of toxin of freshwater ecosystem. This evolutionary and adaptational specificity renders the invertebrates for being ideal model organism for screening of a particular toxin distributed in a definite ecological area. In screening the toxicity of chemicals employing invertebrate species, toxicologists should utilize both *in vivo* and *in vitro* screening systems. Cell culture technology provides an excellent opportunity in screening the toxicity of xenobiotics *in vitro*. However, invertebrate cell and tissue culture is a difficult scientific domain due to variation in the metabolic and other attributes of invertebrate cells. The chemical sensitivity of organisms had been proposed as a primitive character expressed in most all species of major invertebrate Phyla.

Thus, invertebrate in general bears a bright prospect to serve as effective test models for screening the chemical toxicity of xenobiotics. Many of the invertebrates are less researched groups of organisms of inadequate cellular and subcellular information. An in-depth research at the levels of organ, tissue, and cell is required for the proper utilization of aquatic invertebrates as model candidates for toxicity screening.

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