Invertebrate Alternatives for Toxicity Testing: *Hydra* Stakes its Claim

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Summary

The use of vertebrate models in toxicity testing is often challenged for scientific, ethical, and philosophic reasons. Hydra, a freshwater cnidarian, may prove to be useful in this regard. Its simple, transparent, bilayered body allows all its cells to be in contact with the medium, making it a sensitive environmental indicator. The response of hydra to toxicants includes alteration in tentacle morphology, loss of tentacles, change in contractility, altered rate of budding, loss of regenerative capacity, inhibition of gonad formation, inability to attach to substratum, altered behavioral patterns, and mortality indicated by disintegration. Availability of the genome sequence of hydra has expanded the scope of toxicity testing, making this system more versatile. However, the simplicity of this organism, while being its strength, also is its limitation, since its physiology is much less complex than that of vertebrate animals. It is a very good alternative model for preliminary toxicity screening and can substantially reduce the use of vertebrate animals.

Keywords: hydra, xenobiotics, alternative model system

1 Introduction

A wide range of chemicals present in day-to-day life, including petroleum products, petrochemicals, surfactants, pesticides, pharmaceuticals, medicines, household products, food additives, agricultural run-off, by-products of farming, industrial wastes, etc., are released into the environment in large quantities. However, very little information is available about their effects on living and non-living components of the environment. For instance, several studies have shown accumulation of pesticides in groundwater in the original form (Zaki et al., 1982; Gooddy et al., 2001) or in the form of their equally or more toxic end products (Kolpin et al., 2001). A large group of chemicals derived from petrochemical sources have been reported in the ground water (Chen et al., 2000). Many pharmaceutical drugs have been detected unchanged or only slightly metabolized (Heberer, 2002) at low concentrations in sewage effluent and ground water and even in drinking water (Halling-Sørensen et al., 1998; Ternes, 1998). Around 80-100 pharmaceutical chemicals and their metabolites have been measured in both effluent and surface waters in several countries (Fent et al., 2006). Exposure of the public to inadequately tested drugs or environmental agents has resulted in several notable disasters. These include severe toxicity from the use of arsenic to treat syphilis, severe birth defects in children resulting from pregnant women using thalidomide, etc. (Melchert and List, 2007).

2 Assessment of toxicity

Toxicity testing is essential for limiting, if not eliminating, chemical pollution of the environment and associated health hazards. Assessment of contamination can be done chemically. However, their short-term and long-term effects on biological systems are of prime concern for maintaining the health of the environment. Toxicity tests on model animals are conducted to evaluate these chemicals for their potential to cause short-term damages such as maiming or death of the exposed individual or long-term damages including heritable or non-heritable mutations, cancer, birth defects, and other adverse effects.

Action of one toxic agent can be altered to different degrees by exposure to other agents. Different environmental pollutants may interact with each other to generate additive, synergistic, or antagonistic effects. An apparently nontoxic chemical, when combined with another substance, either non-toxic or mildly toxic, may turn into a highly damaging agent. The possibility of interaction of test chemicals with other environmental factors, such as diet, temperature, humidity, other stressors, and infectious agents needs to be considered while assessing their deleterious effects (Gardner, 1979). Further, the extent of toxicity of one or a set of chemicals depends on factors such as the concentration of the toxicant, properties of the toxicant, stability and bioavailability of the chemical, exposure time, and such environmental conditions

as temperature and pH of the medium, as well as susceptibility of the exposed organisms to the toxicant (Babich and Stotzky, 1983).

3 *In vitro* and *in vivo* toxicity testing: Advantages and limitations

Measuring the extent of toxicity can be done *in vitro* or *in vivo*. *In vitro* assays are fast, cheap, and ethically more acceptable alternatives to animal tests. Miniature *in vitro* systems are used to generate quantitative data for the dose that produces a certain biological effect, but their use in predicting *in vivo* effects is still limited. The exact concentration of the contaminating chemical to which cells are exposed *in vivo* is difficult to assess. Moreover, the amount of test compound added to an *in vitro* test system may not be the bioavailable amount in an *in vivo* test system, and it can vary between test conditions and test compounds depending, for example, on the total cell number, the total protein content in the test medium, and the volatility and hydrophobicity of the compound. Serum content of the cell culture medium is known to vary between laboratories, and this can lead to a different outcome of the same test in different laboratories (Clemedson et al., 2003). Such a variety in outcome complicates a comparison of the damaging potency of a biologically active chemical (Heringa et al., 2004). Due to their low cost and sensitivity, *in vitro* toxicity tests are beginning to be used more widely as exploratory tools in toxicity studies. *In vitro* bioassays have been used to investigate the toxic properties of homogeneous particle mixtures, including residual oil, fuel ash, urban air particles (UAP), inert titanium dioxide, elemental carbon, and diesel particles (Carter et al., 1997; Long et al., 2001). Although *in vitro* cytotoxicity assays have been used for many years, these data have been utilized only to a small extent in drug development because of their limited capacity to predict *in vivo* toxicity (McKim et al., 2005). *In vitro* assays have been proven to be valuable for preliminary screening of pollutants with possible biological effects that need to be ascertained in bioassays (Long et al., 2001; Monn and Becker, 1999).

Most reliable data regarding *in vivo* toxicity is obtained primarily in three ways: by the study and observation of people during normal use of a toxic pollutant or from accidental exposures, by experimental studies using animals, and by studies using cells (human, animal, plant). Among these, human studies are the ideal, but they are very challenging and are limited. Knowledge of the toxicity of xenobiotics to humans is derived by three methods: clinical studies where chemicals are administered to human beings under strictly controlled conditions, epidemiological studies that involve observation of people who are accidentally exposed to chemicals during their life or occupation, and through adverse reaction reports from clinicians in volunteers or patients using the drugs. Generally, toxicity found in animal studies is expected to occur with foreseeable frequency and severity in humans. Differences need to be determined with clinical tests in humans. Animal tests for

toxicity are conducted prior to human clinical investigations as part of the non-clinical laboratory tests of pharmaceuticals. Moreover, not all chemicals can be tested in humans; e.g., pesticides and industrial chemicals. In such cases, animal test results often represent the only means by which toxicity in human accidental exposure can be effectively predicted.

4 Selecting a model system

Advantages of animal tests include precisely controlled chemical exposure, well-controlled environmental conditions, and a wide range of toxic effects that also can be evaluated as an ease study of mechanisms of toxicity. Selection of species for toxicity testing depends on the toxicity test to be performed. No single species of animal is sufficient for all toxicity tests. Moreover, it may not be possible to use the most suitable animals, such as primates, for testing because of animal welfare or cost considerations. For example, use of monkeys and dogs is restricted to special cases, even though they represent the species that may react most similarly to humans. Rodents and rabbits are the most commonly used laboratory species due to their availability, low costs in breeding and housing, and comparatively higher predictability of risks to humans from the results obtained. To make the results more reliable, the route_of exposure is kept comparable to that of human exposure. The age of test animals should relate to that of humans. Young adults, newborns, or pregnant animals are used depending on the human population that is at potential risk of exposure to the chemical in question. Dose levels normally are selected so as to determine the threshold as well as the dose-response relationship. Usually, a minimum of three dose levels is used. Toxicity can be measured at different endpoints. LC_{50} (lethal concentration for 50% of the test population) and EC_{50} (effective concentration at which 50% of individuals are dead) are measures of the severity of toxicity. Chronic toxicity is measured in terms of LCx/ECx, which is the concentration lethal for a small percentage showing an effect of x percent; while LOEC is the lowest observed effect concentration, and NOEC is no observed effect concentration. These measures of toxicity allow one to identify the mode of toxicity and its possible amelioration.

Toxicity effects are measured at various levels. These include:
- *Acute Toxicity*: Generally the first tests conducted. They provide data on the toxicity from a single brief exposure.
- *Subchronic Toxicity*: Used to estimate the toxicity from repeated exposures for up to several months.
- *Chronic Toxicity*: Employed to find the toxicity from exposure for a long duration. Tests can be carried on for years, and they involve larger numbers of animals per test.
- *Reproductive Toxicity*: Intended to determine the effects of substances on gonadal function, conception, birth, and growth and development. This uses an even larger number of animals and can extend longer than chronic testing.

– *Developmental Toxicity*: Developmental toxicity testing detects the potential of substances to produce embryotoxicity and birth defects.
– *Carcinogenicity*: Carried out to test the potential of a chemical to cause cancer.
– *Genetic Toxicity*: Genetic toxicity is determined using a wide range of test species including whole animals, microorganisms, and mammalian cells. A large variety of tests have been developed to measure mutations and chromosomal damages.

5 Why and how of ethics and possible remedies

All these approaches involve exposure of animals to a test agent and observation for signs of toxicity for a specific period of time. If toxicity is produced before the end of the scheduled study, it may result in animals experiencing pain and distress as a result of localized tissue damage such as eye or skin irritation or systemic toxicity involving damage to various tissues and organs. Pain and distress also may result from the development of neoplastic and chronic disease, as well as from the development of infections in unprotected animals during vaccine testing. For these reasons, the use of animals in toxicity testing has been the subject of ethical concern for many years, most notably in toxicology and biomedical studies. Inflicting suffering on animals solely to benefit humans poses an ethical dilemma. One way to address these concerns before conducting animal testing is to first assess whether the purpose of the experiment justifies the use of animals. If the purpose is found to be justified, the likely pain, distress, and suffering that might be caused to the animals needs to be assessed. These concerns focus on the use of vertebrates in toxicity studies, since these animals are considered sentient beings.

The principles of Replacement, Refinement, and Reduction of the use of animals in research (popularly known as 3Rs of animal testing) (Russell and Burch, 1959) are guiding principles for the use of animals in research to ensure that the suffering of the model animal is reduced to the barest minimum, if not eliminated.

Replacement: The use of non-animal methods such as cell cultures, human volunteers, and computer modeling instead of animals to achieve a scientific aim. If animals cannot be replaced at all, sentient animals should be replaced with non-sentient animals or less sentient ones.

Reduction: The use of methods that enable researchers to obtain comparable amounts of information from fewer animals, or more information from the same number of animals.

Refinement: The use of methods that alleviate or minimize pain, suffering, or distress, and methods that enhance animal welfare for those animals that cannot be replaced.

Different strategies can be followed to implement the principles of the 3Rs. These include:
a) Avoid unnecessary repetition of experiments and use alternatives such as online databases.
b) Use mathematical and computer modeling, e.g., molecular modeling for drug design, predicting biological activity as well as possible side effects of the drug; pharmacokinetic modeling for predicting effects of drugs/xenobiotics and their metabolites by integrating species-specific physiological parameters; partition co-efficient of the chemicals and metabolic parameters.
c) Use *in vitro* methods, including sub-cellular fractions, tissue-slices, cell suspensions and perfused organs, and cultured tissue from human and other sources in toxicity testing, and for preliminary screening.
d) Use lower organisms with limited sentience and/or not protected by legislation controlling animal experiments. These include such invertebrates as *Drosophila*, *Caenorhabditis elegans*, earthworm, hydra, plants, and bacteria (e.g., *Salmonella*). Some of these can be used as a pre-screen system, especially for agrochemicals and environmental pollutants, genotoxins, and endotoxin detection.
e) Use early developmental stages of vertebrates such as frogs, chickens and mammals for detecting reproductive toxicity, teratogenicity, as well as to study mechanisms of teratogenesis.
f) Use human tissues and volunteers, wherever possible, to avoid the problem of inter-species extrapolation from animals to humans.

6 Hydra as a potential alternative model system

In this context the term alternative refers to any technique that replaces the use of animals, reduces the need for animals in a particular test, or refines an existing technique to reduce the suffering endured by the animal (Rowan and Goldberg, 1985). Replacement of one species with another, particularly a vertebrate with an invertebrate, is considered to be a better alternative. Invertebrates are less likely to raise societal concern compared to higher or even lower vertebrates. When microorganisms, cultured cells and tissues, and *in vitro* methods are unsuitable replacements for animals, invertebrate models are preferred. For instance, use of a lysate of horseshoe crab amoebocytes is simpler, more rapid, and more sensitive than the corresponding vertebrate test involving the rabbit for pyrogenicity testing.

Invertebrates have been used for decades in acute and chronic toxicity tests for hazard identification. Invertebrates can be very efficient screening systems, even though they cannot entirely replace vertebrates in toxicity testing because barriers and diversity in physiology, biology, and genetics in processing different chemicals and stimuli have not yet been overcome.

Hydra, a simple metazoan animal found in clean slow-moving fresh waters, is very useful in toxicity assessment (Karntanut and Pascoe, 2000). It belongs to phylum Cnidaria, class Hydrozoa and has a simple cylindrical body, a head with hypostome, and a mouth surrounded by tentacles at one end, and a foot and basal disc at the other end (Campbell, 1967) (Fig. 1). The body wall

of hydra consists of two cell layers, ectoderm and endoderm, separated by a collagenous acellular layer called the mesoglea. Epitheliomuscular cells are the main structural components of the body wall. In between the epithelial cells, all the other cell types, including nerve cells and the totipotent stem cells called the interstitial cells or I-cells are located. I-cells occur in the gastric region only. They can give rise to nerve cells, nematocytes, gland cells, mucous cells, and, in the sexual cycle, to oocytes and sperm cells. The nerve cells are organized in a nerve net with condensations to ganglion-like structures in the head and in the foot region (Bosch and David, 1987). Developmental processes are permanently active in hydra, as the cells of the body column continuously divide and get displaced toward the extremities, namely, the head/tentacles and the foot. Here, they differentiate in a position-dependent manner and eventually are shed (Bosch and Fujisawa, 2001; Steele, 2002; Bosch, 2003; Bode, 2003). Hydra is considered to be a good model to assess the toxic effects of water pollutants since it is very sensitive to xenobiotics and metal contaminants present in the medium. In addition, the low costs, efficiency, ease of assay, and availability of large clonal populations permit it to be an effective tool for risk assessment in toxicological studies.

Hydra has an unlimited capacity to regenerate lost body parts (Bosch, 2007). Dissociated cells, when recombined, can self-organize and form a normal, fully intact polyp within two days (Gierer et al., 1972). Under normal conditions individual adult polyps do not increase in size despite continuous cell division in the middle regions and migration to either ends, since growth is perfectly balanced by loss of tissue in the form of buds in the lower gastric region and by shedding of cells at the ends of the tentacles and the basal disk. This combination of uniform growth and local cell loss leads to continuous movement of tissue up the body column into the tentacles or down into the buds and basal disk. Hydra has an asexual mode of reproduction by budding. Its constantly active patterning processes are due to the presence of three continuously dividing tissue-specific types of stem cells, viz., ectodermal and endodermal epitheliomuscular cells and interstitial stem cells. Budding appears to be a survival mode since hydra reacts to environmental stressors such as UV through an enhanced rate of budding (Ghaskadbi et al., 2005). Under certain conditions, hydra can produce gonads (Fig. 1) and undergo sexual reproduction.

7 Toxicity testing using hydra

Since all the cells of hydra are distributed in only two layers, every cell is constantly bathed in the surrounding medium and exposed to the immediate environment. This makes hydra very sensitive and susceptible to minute amounts of environmental toxicants. Studies in the Indian species of the hydra *Hydra vulgaris* Ind-Pune (Reddy et al., 2011a) show that it takes only a few minutes to alter the cytoskeleton and cell surface features of hydra by exposure to taxol and cytochalasins (Ghaskadbi and Mulherkar, 1984; Chaugule and Ghaskadbi, 2006). Because of
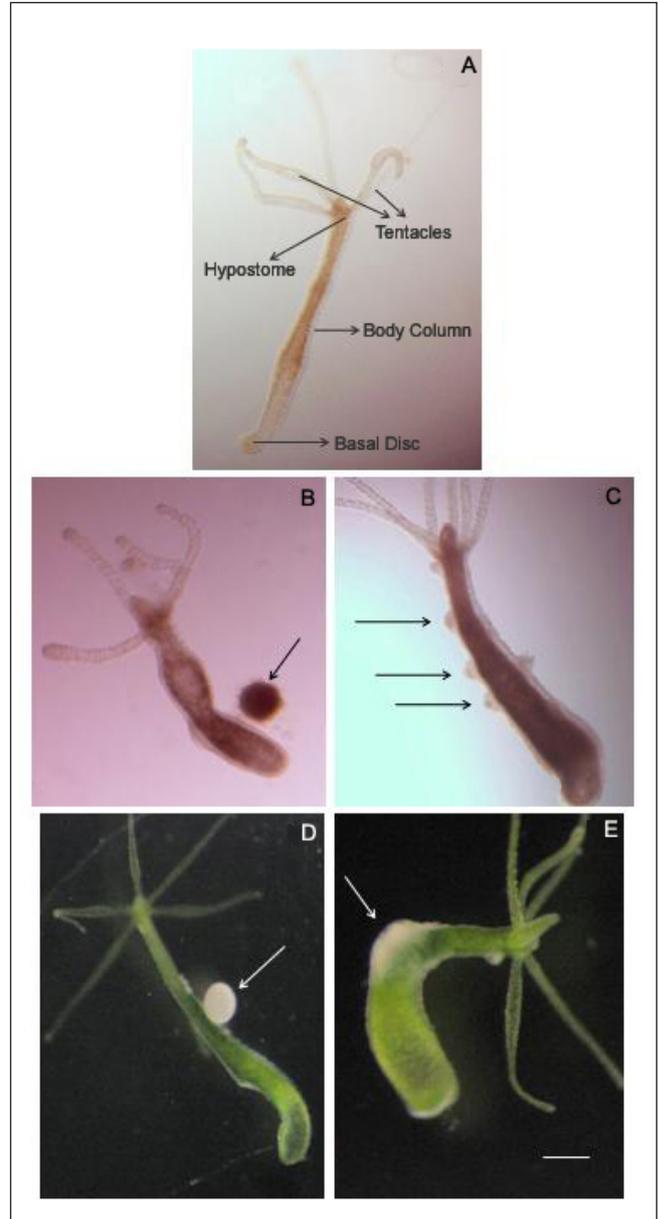

Fig. 1.: (A) Photomicrograph of a live specimen of Hydra vulgaris Ind-Pune with its major body parts labeled. (B) Photomicrograph of a live specimen of *Hydra vulgaris* AEP strain with an oocyte (arrow). (C) Photomicrograph of a live specimen of *Hydra vulgaris* AEP strain with multiple testes (arrows). (D) Photomicrograph of a live specimen of *Hydra viridissima* with a single oocyte (arrow). (E) Photomicrograph of a live specimen of *Hydra viridissima* with a testis (arrow). The green color of *Hydra viridissima* is due to the symbiotic unicellular alga Chlorella. Scale bar = 1 mm.

the ability of hydra to asexually produce a clonal population rapidly through budding, it is possible to have a large population available for testing, making the bioassay highly reproducible. Ease of maintenance, simplicity of assay, and high reproducibility have contributed to the popularity of hydra as model organism in acute and chronic toxicity tests of water-soluble compounds (Lum et al., 2003).

Hydra has long been used in classical ecotoxicological testing. The rapid rate of asexual reproduction of hydra by budding allows the effects on population growth of a potential toxicant to be determined in the laboratory. It also provides a rapid, sensitive, and precise approach to the measurement of environmental pollutant effects on freshwater invertebrates (Stebbing and Pomroy, 1978). Hydra is known to be sensitive to both metal and organic contaminants (Slooff et al., 1983). Deleterious effects on morphological features of hydra were noted due to exposure to the heavy metal pollutant cadmium at ppm concentrations (Kar and Aditya, 2007). This study also provided a random scale to measure the extension of pollution of fresh water in an outdoor setting. In a unique study, the advantages of symbiosis were revealed when endosymbiotic green hydra (*Hydra viridissima*) and asymbiotic brown hydra (*Hydra oligactis*) were exposed to aluminium sulphate. Aluminium toxicity triggered mortality, morphological, behavioral, and DNA damage in both the species. DNA damage was greater in brown hydra than in green hydra, however, while behavioral responses to the presence of aluminium ions were observed more rapidly in green hydra. The toxicity also affected reproduction. Brown hydra was more susceptible to aluminium than green hydra, confirming the evolutionary advantage provided by symbiosis (Kovačević et al., 2007). However, it should be noted that *H. viridissima* was found to be more sensitive to copper and cadmium than *H. vulgaris* and *H. oligactis*, while all of them were equally vulnerable to zinc (Karntanut and Pascoe, 2002). In an effort to validate a protocol using new sublethal endpoints of feeding behavior and the ability to attach to substratum, *Hydra attenuata* polyps were exposed to increasing concentrations of the heavy metal pollutant cadmium chloride. Efficiency of prey capture, ingestion, and attachment impairment were compared to those of morphology and reproduction. These endpoints were much more sensitive to lower concentrations of toxicants than the lethal endpoints. A significant decrease in prey ingestion capacity was seen at lower concentrations of cadmium chloride where capacity to capture prey was unaffected. Ability to attach to the substratum was found to be very sensitive to pollutant concentration, significantly more than reproduction (Quinn et al., 2007). Sensitivity of hydra to extremely minute concentrations (ppm to ppt levels) of organophosphate nerve agents has made it an efficient biosensor. The Hydra bioassay is proposed as a prescreening tool in determining the toxicity of related organophosphorus nerve agents, as well as individual stereoisomers that are yet to be screened for toxicity (Stebbing and Pomroy, 1978).

Dispersants are used for reduction of the toxicity of oil spills to surface animals, birds, and mammals. Along with the oil, however, dispersants themselves were found to be toxic to freshwater organisms (Vindimian et al., 1992). Some of the dispersants were found to decrease the growth rate in green hydra at sublethal concentrations (Mitchell and Holdway, 2000).

In a comprehensive study, acute and chronic toxicity of ten commonly prescribed drugs were assessed using *Hydra vulgaris,* and some of them were found to adversely affect the regenerative capacity on chronic exposure concentrations (Pascoe et al., 2003). In a similar study, eleven pharmaceuticals and their solvents were classified as toxic, harmful, and non-toxic based on acute and chronic exposure assays using hydra. Acute effects involved morphological changes, while chronic effects were measured using feeding behavior, growth, and attachment as parameters (Quinn et al., 2008).

Hydra polyps are reported to be highly sensitive to short-term exposure to endocrine disruptors such as 4-nonylphenol (4-NP) at concentrations normally found in contaminated sites, but not at those concentrations reflecting lower levels of environmental contamination. Within the first hour of exposure to 4-NP at lethal concentrations, apoptosis was induced, indicating rapid effects of the chemical, and at a lower concentration, loss of tentacles and consequent impaired feeding was reported (Pachura et al., 2005). When exposed to drugs that induce oxidative stress, hydra responds with activation of antioxidant defense mechanisms and metabolic pathways (Quinn et al., 2004).

Effects of reproductive hormone-mimetic compounds found as ecotoxicants have been studied in some invertebrates, including hydra. One of these is bisphenol A, a weak estrogenic compound (Krishnan et al., 1993) that is contained in a variety of matrices used for food and drink containers and for dental sealants; it is found in sewage water as well. It interfered with asexual and sexual reproduction of hydra, but only at doses much higher than those detected in freshwater environments (Pascoe et al., 2002; Fukuhori et al., 2005). Data from a hydra regeneration assay was easily and directly correlated with those from vertebrate *in vivo* teratogenicity and, therefore, the hydra regeneration assay is proposed to be an effective screening tool for the teratogenic potential of various toxicants (Wilby et al., 1990).

Hydra has been found to be a suitable model for testing the biological effects of colloidal semiconductor nanocrystals, the latest biocompatible materials being introduced in biology and medicine. Hydra, treated with quantum rods, showed a subtle tentacle writhing activity that was shown to be calcium-dependent. Results from these assays indicated that the interactions between living organisms and newly synthesized nanomaterials need to be more thoroughly investigated before they are employed in any new nanostructure for such biological purposes as cell-tracking studies, drug delivery, etc. (Tino et al., 2011; Tortiglione, 2011).

8 Promise of hydra as a toxicity testing model

The presence of three lineages of stem cells, perpetual and regular replacement and renewal of all somatic cells in the body, and a continuously active developmental program

make hydra virtually immortal. It can reproduce asexually, producing genetically identical populations endlessly under normal conditions and using the sexual mode under unfavorable environmental conditions. This model offers a unique opportunity to study short-term as well as long-term effects of various classes of toxicants on development, growth, normal life processes, and senescence.

Hydra can survive even when interstitial stem cells are completely eliminated experimentally. This model provides an opportunity to test various cancer stem cells, targeting therapeutic agents for their effects on the vital processes of the organism. Similarly, developmental toxicity of various chemical pollutants can be studied in budding, regeneration, and embryonic development. Budding, as well as head- and foot-regenerating middle pieces of hydra, are distinct and unique systems where all the pattern-forming processes are active without the early embryonic cues. This can provide a good model to investigate the effects of teratogenic pollutants that affect patterning in higher vertebrates. Constantly, recycling somatic cells can be the targets of the molecules that induce apoptosis and necrosis, and their modes of action can be studied in this system. Also, any sign of senescence induction in hydra is certainly because of the chemical being tested since there is no natural aging in hydra.

Until now, toxicity related gene-expression studies have been limited mainly to traditional model organisms whose genomes have been sequenced, such as *Drosophila* or the mouse. However, the recently published whole genome sequence of hydra (Chapman et al., 2010) has made it possible to use hydra in innovative ways in toxicity testing. This allows the use of gene expression profiles to identify a battery of toxins that pose a risk to the environment or to human health since it is quicker and cheaper than testing on mammalian models. A large number of hydra genes have been discovered since its genome sequence has become available, including unexpected ones such as the neural tube patterning gene *noggin* (Chatterjee et al., 2001; Chandramore et al. 2010; Chandramore and Ghaskadbi, 2011), the gene for photosensory protein opsin (Plachetzki et al., 2007), the oncogene *Myc* (Chapman et al., 2010), several genes involved in vertebrate development (Frobius et al., 2003; Reddy et al., 2011b; Hoffmeister-Ullerich 2007), and in innate immunity (Augustin et al., 2010). The effects of environmental insults on these genes can be studied to derive insights into risks to humans posed by these toxins.

The generation of transgenic hydra (Wittlieb et al., 2006) has enabled the study of toxicity from a molecular and cellular perspective. The effects of over-expression/knock out of any homologous or heterologous genes can be studied in custom-made transgenic hydra lines. A transgenic hydra line has been developed expressing a FoxO transcription factor that mediates cellular responses to stress, including oxidative stress and dietary restriction (Bridge et al., 2010), and this can be used as an effective model to study the effects of heat shock. This line of hydra can easily be used to study the consequences of exposure to other inducing factors.

9 Vision for the future of toxicity testing

Systems biology, bioinformatics, and rapid assay technologies are paving the way to a better understanding of how cellular networks or pathways in the human body carry out normal functions. When important pathways are significantly altered by chemical exposures it can lead to significant morbidity or mortality. The latest developments in toxicity-testing can elucidate the cellular response pathways that can result in such a condition. Such a system can evaluate biologically significant alterations without relying on studies of whole animals. For the foreseeable future, some targeted testing in animals will need to continue, as it is not currently possible to sufficiently understand how chemicals are broken down in the body using tests in cells alone. These targeted tests will complement the new rapid assays and ensure the adequate evaluation of chemicals. Dose-response and extrapolation can enable assessment of the possible relevance of results to whole human systems. While we strive to minimize the unnecessary use of animals in experimentation by finding ideal alternative toxicity testing methodologies and protocols, hydra is emerging as a very powerful model system for toxicity testing at the organismal, cellular, and molecular levels.

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