Alternatives to Animals in Education, Research, and Risk Assessment: An Overview with Special Reference to Indian Context

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Summary

Animal experimentation remains a matter of debate and controversy. On one side is the opinion that animal use in education, research, and testing raises concerns relating to ethical treatment of animals, the source from which animals are obtained, ill-designed experiments, relevance of inferences from the testing outcomes, safety issues, biodiversity issues, environmental concerns, etc. On the other side is the opinion that the benefits that have come from animal research would not have been realized if not for animal experiments. These concerns led to the 3Rs principles of Replacement, Reduction, and Refinement in the use of animals in experimentation, otherwise known as the concept of alternatives. Once a matter of imagination, today science has evolved such that drugs, cosmetics, and agrochemicals can be tested adopting scientifically validated non-animal methods. Today, the science of alternatives has gained recognition from both an ethical and a scientific point of view. The breakthroughs in ICT, cell culture techniques, in vitro toxicology, molecular tools, functional genomics, tissue engineering, systems biology, etc., have made it possible to take the concept of alternatives from a myth to reality. There is still a long way to go, however: just as science has no end, the search for alternatives has no end.

Keywords: alternatives, in vitro systems, in silico approaches, validation of alternatives

1 Introduction

The use of animals in science is a contentious issue, with opinions from scientists, teachers, animal protectionists, and the public either to support animal experimentation or to reject/minimize it (Pawlik, 1998; Giridharan, 2000; Greek and Greek, 2010; Greek, 2012). The main purposes of animal experiments are for: basic biological knowledge; fundamental medical research; the discovery and development of drugs, vaccines, and medical devices; toxicity testing of drugs, other chemical entities, and consumer products; and education and training (Taylor et al., 2008). These contexts of animal use can be broadly classified into (i) education and training, and (ii) research and testing. It is roughly estimated that among the several million animals sacrificed for the cause of science, in any country, 1-10% are used in education and the rest in research and testing. There has been a movement against the use of animals in endeavors in science on several grounds and, as a result, greater awareness has been generated. A concerted effort across the globe has resulted in a considerable reduction in the number of animals used and increased recognition that animals are sentient beings. It is cause to rejoice that India has made rapid strides in phasing out animal use in education and training, but the scenario with respect to research and testing has changed very little. This article is an attempt to sensitize the stakeholders to the need to promote the most humane science.

2 On the use of animals in education and training in basic sciences and the potential alternatives

In education and training, wild-collected as well as laboratory-bred animals in millions are used in the name of knowledge acquisition and/or skill development in basic life sciences, as well as in professional education for medical, veterinary, pharmacology, etc., purposes. Dissections of and experiments using animals have been practiced as laboratory exercises in zoology and allied life sciences since the 1920s, at a time when the subjects of animal anatomy, taxonomy, and evolution were emphasized in theory courses. These classical subjects have now been relegated to the background in the light of emerging subjects such as biochemistry, molecular biology, and biotechnology, warranting lectures and laboratory exercises in these subjects. Further, the demand for animals for purpose of dissections has increased to such an extent that animals are no longer available in large enough numbers to meet the demand. The large-scale removal of animals from their natural habitats for an unnatural purpose can potentially lead to biodiversity loss and an ecological crisis. Laws have been enacted protecting selected animal species, but these laws are often not heeded, and animals have been continued to be used in education. Social groups and scientists also have questioned both the logic and the relevance of animal dissections in education in the contemporary

scenario. The ethical principle of reverence for life demands that human beings should protect their fellow creatures, the animals, which, like themselves, are sentient beings. It also implies an obligation to respect their dignity and the right of each species to thrive and flourish in its natural manner (Swiss Academy of Medical Sciences, 1995).

Balcombe provided empirical evidence from educational studies to show that in most contexts animal dissection is unnecessary – and even counterproductive – to achieve valid educational goals, especially higher order goals (Balcombe, 2000, 2001). It has been strongly recommended that the new alternative learning modalities arising from information and computer technology (ICT) and other areas can effectively replace dissections and experiments using animals (van der Valk et al., 1999; van der Valk, 2006). A variety of simulation programs have been developed to enhance the learning methodology and to provide students with a better alternative in terms of learning outcomes (Hughes, 2001; Dewhurst, 1994). These software applications typically fall into one of two categories: multimedia presentations or enhanced multimedia presentations. The multimedia presentation category includes: Neotek's Frog Dissection Lab; Operation Frog Deluxe by Tom Snyder Productions (Scholastic); VisiFrog by Ventura Educational Systems; Boreal Labs' Digital Dissection Series; Drylab by Tangent Scientific; Biolab by Carolina; Pro-dissector FROG a Schneider & Morse Group Production, etc. These products offer very similar functionality, presenting a student with text, artwork, photographs, and in some cases, video clips. While they present the student with an integrated and self-paced learning environment, they only offer linear and/or restricted learning paths, and every student ultimately observes the same thing, usually in the same sequence. The enhanced multimedia presentations include: Digital Frog by Digital Frog International; CyberEd Dissection Series by Plato; DissectionWorks by ScienceWorks; and Frogguts by Frogguts, Inc. These products allow a student to select a tool, such as a scalpel, and float the tool over a 2D representation of a frog. Clicking the mouse in the right place triggers a video clip or computer animation of the frog being cut. The student does get the benefit of controlling the dissection and seeing content in context, but the limitation here is that these products offer no physical simulation of tissue being cut or manipulated but only pre-recorded renderings and, again, every student sees the same sequence or set of images, texts, and videos. V-Frog™, a more recent addition to this list, is an innovative virtual reality-based dissection simulator developed using virtual surgery technology[1], and it is highly efficient in achieving quality learning (James, 2010). The Center for Alternatives to Animal Testing (CAAT) organized a workshop in which specialists from different countries met and set up a framework for a comprehensive educational program, and a consensus was agreed upon for generating teaching materials pertaining to the 3Rs approach in education (Daneshian et al., 2011).

Given that many countries have dropped dissection of animals from the curriculum, the pace of change in India has been initially rather slow (Akbarsha, 2007; Venkatesha, 2007; Vasudevan and Supriya, 2011), although animal welfare groups and teachers from India have been demanding discontinuation of animal dissections in education. The Central Board of Secondary Education (CBSE), constituted by the Union Government of India, a Board of Education for public and private schools, stopped prescribing dissections in its curriculum, the first dramatic change in this connection in India. Gujarat, Haryana, Uttar Pradesh, West Bengal, Rajasthan, Chhattisgarh, Himachal Pradesh, and Tamil Nadu, to mention a few, also dropped dissections from the school curriculum of the respective State Boards. Mahatma Gandhi-Doerenkamp Center (MGDC) for Alternatives to Use of Animal in Life Science Education, located at Bharathidasan University, Tiruchirappalli[2], which draws strength from a number of non-governmental organizations, is forging ahead with a movement in the country to curtail animal use in higher education (Akbarsha and Pereira, 2010; Akbarsha et al., 2010). The University Grants Commission (UGC) has been regularly reviewing the use of animals in life science education and, recently, brought up guidelines for discontinuation of dissection and animal experimentation in zoology/life sciences education in a phased manner[3], a progressive effort in this direction. Implementation of these guidelines, and the change from "dead zoology" to "live zoology", i.e., learning about the animals without removing them from their natural habitats, is imperative. Allowing the learner to visit the animals and make observations, will not only save the millions of animals being killed/sacrificed for purpose of higher education in India, but also will facilitate the introduction of ICT tools, the "alternatives", in the pedagogy of laboratory exercises. Thus, India today can be proud of being the pioneer in formulating guidelines of this kind for other countries to adopt. Much depends on to what extent these guidelines will be adopted by the universities and colleges. This apprehension remains because there are still orthodox institutions, stubborn teachers, and old-fashioned academic bodies that are reluctant to change to the innovative pedagogy.

3 On the use of animals in education and training in biomedical sciences/pharmacology and the potential alternatives

Medical education in India previously required the use of animals such as frogs, rats, guinea pigs, dogs, etc., as tools for learning first-hand certain aspects of anatomy, physiology, biochemistry, pharmacology, surgery, etc. This use, from a global perspective, has emphasized two components – the acquisition of knowledge and the development of skills (Balcombe, 2000). It is a fact that this use has done well in developing skilled clinicians, surgeons, pharmacologists, and so on. However, those were the

[1] http://www.tactustech.com/vfrog/
[2] http://www.mgdcaua.org/
[3] http://www.ugc.ac.in/oldpdf/commissiondecision/479.pdf

days when there were fewer institutions, fewer students, ethical concerns were rarely raised – or not raised at all – and no better learning/skill development modalities were available. With the increased number of institutions, increases in enrolment, and the recognition of animal sentience and the consequent ethical considerations, combined with the development of several "alternatives" through innovative technologies have resulted in a paradigm shift in the use of animals in medical education in Europe and the USA. The alternatives include the mannequins, simulators, virtual settings, human volunteers, self-experimentation, etc. Special mention should be made of the successes made using virtual reality simulators (VRS), human patient simulators (HPS), and minimally-invasive surgery (MIS) (Seymour et al., 2002; Good, 2003; Gallagher, 2005; Seymour, 2008). Studies have proved that learning and/or skill development adopting these various "alternatives" not only compensate for the real-time exposure to animal models, but the outcomes are much better (Dewhurst, 1994; Hughes, 2001). However, the pace of change in India has been rather slow (Roy and Tekur, 2001; Hariharan, 2004; Dhingra et al., 2006). The curricula still prescribe laboratory exercises using animals, and the training is mandatory, in spite of the recent liberal permission from the Medical Council of India (MCI) and the Pharmacy Council of India (PCI) that "alternatives" can replace animals in laboratory exercises. The conservatism in the Indian mind-set has precluded, by and large, change to the modern and novel approaches in medical and pharmacy education. Concerted effort from MGDC, a few non-governmental organizations, and enlightened teachers should lead to a change to "alternatives," and a desirable change is expected soon.

4 Use of animals in drug development and risk assessment

Animal experimentation remains a matter of debate and controversy. Those who are not in favor of animal use in drug discovery and risk assessment raise concerns relating to ethical treatment of animals, the source from which animals are obtained, ill-designed experiments, relevance/inferences of the testing outcomes, safety issues, etc. Those who are in favor animal testing point out the benefits that have come from animal research, such as organ transplants, open-heart surgery techniques, lifesaving drugs, effective insulin and cancer treatments, and animal and human vaccines. One cannot deny the contributions made by animals for the betterment of humans, but it should be realized that animal testing is not the gold-standard, and accurate prediction of drug toxicity on the human remains a major challenge in drug development (Li, 2004). Over the past decades, a number of drugs have been withdrawn due to adverse effects, sometimes fatal, on humans, despite having been declared safe after having passed all the animal tests required by the Regulatory Authorities. The cost of withdrawal of a drug from the market is astronomical, including losses in resources and time spent in drug development. It has been estimated at $ 800 million, with an average development time of 12-15 years required for a successful drug (Dimasi et al., 2003). Again, this cannot be considered as a gold standard (Morgan et al., 2011), as the development cost depends on a variety of factors (Dickson and Gagnon, 2004). A study reveals that out of 10,000 new chemical entities (NCE), only one may become a successful drug and, according to US Food & Drug Administration (FDA) statistics, only around one in every ten new medicinal products that progress to clinical trials ever reaches the registration stage[4]. Unfortunately, some of the drugs have caused horrific harm to people, even though they had all been "safety tested" on animals. *Opren*, hailed as a "wonder-drug" for arthritis, was withdrawn in 1982 after 62 deaths and 3,500 serious side effects in the UK alone, including damage to skin, eyes, circulation, liver, and kidneys[5]. *Practolol*, a selective beta blocker marketed under different names, was one of the biggest drug disasters, and it was withdrawn after reports of adverse drug reaction, including 40 deaths (Abraham and Davis, 2006). The famous case of thalidomide, administered to pregnant women as a harmless tranquilizer, caused innumerable birth defects worldwide. In December, 2004, *Vioxx*, the blockbuster drug from Merck & Co. Inc., was withdrawn from market due to serious cardiovascular events, including heart attacks and strokes (Mukherjee et al., 2001; Juni et al., 2004; Topol, 2004). But its withdrawal came too late, as 80 million patients had taken this medicine, with a 38,000 estimated dead (Juni et al., 2004). Had this not been reliant on animal testing, such disasters could have been avoided[6]. In fact, there is a long list of drugs that have been recalled – drugs that are withdrawn from the market and drugs for which safety alerts have been issued, either by US FDA or by other regulatory agencies[7,8,9]. (e.g., Wysowski and Swartz, 2005)

About 70 million chemicals have been synthesized, as registered in Chemical Abstracts Service. It is not really clear how many of these chemicals are found in consumer products, in the environment, or in our bodies. A reasonable estimate is that people are exposed to about 100,000 relevant synthetic chemicals, in contrast to the 5,000 to 10,000 for which actually (widely varying in depth) safety assessments exist. However, we are most likely exposed to an even larger number of chemicals, given all the naturally occurring sources (Hartung, 2010). The conventional method of assessing these chemical entities for toxicity, or subjecting them to safety and risk assessment, is to do animal tests. A variety of tests are conducted on a variety of animal species. Over and above the animals used in the preliminary screening, chronic toxicity testing, and risk assessment (hazard identification, hazard characterization, exposure assessment, and

4 http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/CriticalPathOpportunitiesReports/ucm077262.htm
5 http://www.dlrm.org/resources/alternative.htm
6 http://www.pcrm.org/search/?cid=1172
7 http://www.fda.gov/safety/recalls/default.htm
8 http://www.fda.gov/ohrms/dockets/98fr/100898b.txt
9 http://www.medindia.net/patients/patientinfo/drugs-banned-in-india.htm

risk characterization), the chemical is to be assessed for ADME, and then there is a battery of tests that need to be conducted for safety evaluation. These include carcinogenicity, teratogenicity, genotoxicity, reproductive toxicity, etc. (Blaauboer, 2002). The total animal requirement to subject all chemicals likely present in the environment, consumer products, and foods thus will be astronomical; to that extent, animals are subjected to pain, distress, and sacrifice. Added to this is the problem that the data generated in animal models do not fit well for the human context in view of species differences in metabolism of the drug/putative toxicant. The major issue here concerns the Phase I and Phase II metabolizing enzymes, more importantly Cyp450 isoenzymes, which differ between species (Bibi, 2008; Graham and Lake, 2008). An estimate made by Taylor et al. (2008) puts the global use of animals in experiments during 2005 alone at a little more than 115 million.

This only reiterates that it is important for scientists to make their choices with a scientific rationale, a sense of compassion and ethics regarding animal use, and then to look at the need/possibility of using better, more humane and more precise tools (Lord, 2004; NRC, 2007; Yang, 2009). Some of the pertinent questions in this connection are i) does research and testing really require this many animals; ii) can animal testing be conducted in a way that does not subject the animals to distress or pain; and iii) can testing be done without animals?

5 The 3Rs principles

It was precisely these three questions that led to the 3Rs principles of Replacement, Reduction, and Refinement in the use of animals in experimentation, otherwise known as the concept of "alternatives." Science has evolved such that drugs, cosmetics, and agrochemicals now can be tested using scientifically validated non-animal methods, thereby saving/protecting the lives of human beings and also saving the lives of several million animals from pain/distress and/or death. The alternatives have paved the way for better, more precise and more credible scientific investigations. In addition to the animal welfare aspects, alternatives offer advantages such as cost-saving and higher throughput.

The concept of 3Rs was propounded in the book "*The Principles of Humane Experimental Technique*" by the British scientists W. M. S. Russell and R. Burch (1959). The word "alternative" was coined by D. H. Smyth (Smyth, 1978) to describe the 3Rs. Russell and Burch's book seriously challenged the scientific community about the use of animals in research. The Declaration of Bologna in 1999 further reiterated the need and rationale for the scientific community to adopt alternatives[10]. Today, the science of alternatives has gained recognition from both ethical and scientific points of view. The breakthroughs in *in vitro* systems, *in silico* toxicology, molecular biological tools, omics approaches, tissue engineering, etc., have made it possible to take the concept of alternatives from the perspective of animal testing from myth to reality (Aardema and MacGregor, 2001; Kniewald et al., 2005; Bhogal et al., 2006; Kroeger, 2006; Chapin and Stedman, 2009; Hartung and Hoffmann, 2009; Elliott and Yuan, 2010; Roggen, 2011).

6 *In vitro* systems: alternatives to animal testing in risk assessment

Since alternatives to animal testing rely heavily on *in vitro* systems, albeit with some limitations (Roggen, 2011), it is pertinent to highlight some of the assays/discoveries that redefined the risk assessment process and, eventually, set the stage for more challenging research (Eisenbrand et al., 2002; Bernauer et al., 2005). Application of *in vitro* techniques has resulted in a sincere effort to find test methods capable of screening large numbers of chemicals so as to reduce animal testing and, at the same time, facilitate risk assessment strategies (Bal-Price, 2008; Coecke et al., 2007; Lein et al., 2007; Crofton et al., 2011). One of the most successful alternative tests has been the Limulus Amoebocyte Lysate assay (Pearson et al., 1985), which was developed as an alternative to the rabbit pyrogen test, saving one million rabbits annually. More recently, the human whole blood pyrogen test has proved to be a better means of finding pyrogenicity than the other tests (Hartung et al., 1998). Cell-based assays have been developed to evaluate the toxicity of compounds, and accurate, precise, and sensitive end-points have been introduced. Various cultures of brain cells have been used to deliver insights on CNS disorders and elucidate the pathogenesis of oxidative stress in neurodegenerative brain disorders, AIDS-associated brain pathology, drug abuse, and aging (Aksenova et al., 2005). Neuroblastoma or glioma cell lines can be used to investigate the interaction of neurotoxicants with ion channels or receptor and signal transduction systems, as well as with basic metabolic functions. Several neuroblastoma or glioma cell lines or PC12 cells have been used extensively in neurobiological studies, thus providing a large amount of information on their physiology and biochemical composition. Mouse neuroblastoma cell lines have been used in screening teratological compounds (Mummery et al., 1984) and in predicting acute toxicity of certain groups of substances (Walum and Peterson, 1983). Cultures of Schwann cells may represent a good model to study the effect of chemicals on myelination. However, according to Veronesi (1992), because of the complexity of neurons and the lack of a cell line to mimic the *in vivo* neuronal conditions, no single *in vitro* preparation can be relied upon to detect all possible end points. However, many studies are underway, and at least one study should prove promising. Scientists have developed a three-dimensional brain cell culture system that resembles brain tissue in many of its characteristics. This means that various aspects of ischemia research now can be investigated in the test tube instead of in animals. Due to its significance, this *in vitro* method was evaluated by the EU ACuteTox Project as a

[10] http://www.icare-worldwide.org/indian_congress/bologna.html

model for identifying neurotoxic chemicals without laboratory animals[11]. A rapid, reproducible, and sensitive neurotoxicity testing platform that combines the benefits of neurite outgrowth analysis with cell patterning, known as network formation assay (NFA), has been recently developed. This approach involves patterning neuronal cells within a hexagonal array to standardize the distance between neighboring cellular nodes and thereby standardize the length of the neurite interconnections. This feature, coupled with defined assay coordinates, provides a streamlined display for rapid and sensitive analysis. This assay has the potential to meet the demands of high-throughput applications in neurotoxicology and neurodevelopmental biology (Frimat et al., 2010).

In vitro genotoxicity assays based on the detection of mutagenicity in bacteria or chromosomal damage in mammalian cells have been useful in evaluating genotoxic, non-genotoxic, carcinogen or non-carcinogen compounds. The *in vitro* micronucleus assay is a mutagenicity test system used for the detection of chemicals that induce the formation of small membrane-bound DNA fragments such as micronuclei in the cytoplasm of interphase cells (Fenech, 2000). In recent years, scientists increasingly have seen the *in vitro* micronucleus test as an attractive tool for genotoxicity testing because of its simplicity and wide applicability in different cell types (Decordier and Kirsch-Volders, 2006). The assay is a potential method for genotoxicity/photogenotoxicity screening of drug candidates owing to reduced false positive results, and it requires a lesser amount of toxic compound (Witte et al., 2007). Moreover, the assay has been endorsed by ECVAM and approved by REACH for regulatory use as part of the genotoxicity test battery (ECVAM, 2006a), demonstrating the authenticity of the assay.

The study of carcinogenesis has been greatly facilitated by the discovery of *in vitro* morphological transformation of mammalian cells in culture. The most frequently used endpoint for cell transformation is morphological transformation, which involves phenotypic alteration in the cultured cells, such as alterations in cellular morphology, disorganized patterns of colony growth, and acquisition of anchorage-independent growth (Barrett et al., 1986). The most commonly used cell transformation assays are Syrian Hamster Embryo (SHE) assay, Balb/c assays, and C3H/10T1/2 assay. A study reveals the Balb/c assay as competent to evaluate the carcinogenic potential of chemicals and environmental mixtures. It is very likely that, on validation, it will replace animal testing in a two-stage cell transformation similar to two-stage carcinogenicity assays *in vivo* to detect both tumor promoters and weak initiators (Vanparys et al., 2010). The replacement of the classical acute toxicity test, or LD50, with the Acute toxic class method / Fixed dose procedure / Up-and-down procedure, as approved by OECD, can reduce animal use from 45 to 8 animals per test chemical, an illustrative example for reduction and refinement alternatives.

The Bovine Corneal Opacity and Permeability (BCOP) eye test has been validated by ECVAM and accepted as a screening test to detect ocular corrosives and severe irritants in a tiered testing strategy (ECVAM, 2007). The test substance is applied to isolated bovine cornea (obtained from slaughterhouses) and determination of irritation or corrosion is evaluated by measuring changes in opacity and permeability using an optical instrument. Isolated chicken eye (ICE) or chicken enucleated eye test (CEET) also has been approved by ECVAM. All these tests are organotypic *in vitro* approaches to animal testing, and it has been estimated that there will be at least 10% reduction in the number of animals killed in the US alone.

Engineered skin/epithelial models have come to replace the animals in the skin sensitization/irritation/corrosion testing of cosmetics (Alder et al., 2010). A team of scientists working at L'Oréal, France, created a reconstructed skin model that has been validated by ECVAM (ECVAM, 1998) and approved as a replacement or refinement to animal testing for skin corrosivity. The product, marketed under the name of EPISKIN in Europe, is a huge success, both in terms of saving thousands of innocent animals and as a significant step forward in the field of science. The model is created on a base designed on human collagen on which adult keratinocytes are layered to get a 3D structure. The cells grow, divide, and then differentiate in the air-exposed culture. At the end, the epidermis consists of a mitotic layer, a mucous Malphigian layer and a functional horny layer. The test substance is either applied topically or in the culture medium, and the irritancy is identified by MTT assay. Studies have shown the reproducibility and reliability of EPISKIN in assessing toxicity of cosmetic and dermatological products (Roguet et al., 1994, 1998; Faller et al., 2002). Scientists are working to test the applicability of EPISKIN to examine whether reconstructed skin can be useful for prediction of the genotoxic effect of a compound (Flamand et al., 2006; Ouédraogo et al., 2007). The study was extended to discover whether this model can prove to be an effective barrier to toxic compounds. A specific co-culture system using target cells (lymphoma cells) cultured underneath the *in vitro* reconstructed skin (EPISKIN) was developed and a micronucleus assay was performed. The results concluded that EPISKIN is a metabolically competent tissue and a biological barrier mimicking realistic conditions of use (Flamand et al., 2006; Ouédraogo et al., 2007).

Similarly, another reconstructed skin model, called EpiDerm, has been validated (ECVAM, 2000a). It closely parallels human skin, thus providing a useful *in vitro* means to assess dermal irritancy and toxicology (Cannon et al., 1994). It possesses a human 3D skin-like tissue structure with a complete stratified epithelium (Kandárová et al., 2007) and *in vivo*-like lipid profile (Ponec et al., 2002). Recent studies indicate that EpiDerm reproduces many of the barrier function properties of normal human skin. SkinEthic and CORROSITEX, the other models, developed on the same lines, have been validated and approved by the regulatory authorities (ECVAM, 2006a,b). Skin sensitization is an immunologically-mediated cutaneous reaction to a substance. The classical Local Lymph Node Assay (LLNA) is an alternative method. The principle behind this test is that sensitizers induce a proliferation of lymphocytes in

[11] http://www.acutetox.eu/

the lymph node draining the site of chemical application (For more information on alternative methods, the reader may visit http://altweb.jhsph.edu).

7 *In vitro-in vivo* approach in drug testing and risk assessment

One of the major questions raised in connection with *in vitro* toxicology is that a cell does not represent the human body in its holistic perspective, i.e., cells of different kinds in tissues and organs, and therefore cell-cell interaction and multiple targets are not mimicked. Physiological situations where one organ doing the metabolic function and another organ responding to the metabolite(s) is not provided for in the conventional *in vitro* tests. It is known that species differences form the basis for the failure of several drugs. A large number of drugs and other chemicals have been shown to invoke the action of hepatic microsomal cytochrome P450 (CYP) isoforms in experimental studies (Bibi, 2008). Most CYP forms are induced by receptor-mediated mechanisms leading to an increase in gene transcription. Important nuclear receptors involved in the induction of CYP1A, CYP2B, CYP3A, and CYP4A subfamily forms comprise, respectively, the aryl hydrocarbon receptor, the constitutive androstane receptor, the pregnane X receptor, and the peroxisome proliferator-activated receptor alpha. Hepatic CYP form induction can be assessed by *in vivo*, *ex vivo*, and *in vitro* methods. Significant species differences can exist in the enzyme induction response to a given chemical and also in the toxicological consequences of induction. Hepatic CYP form induction in humans may lead to clinically important drug-drug interactions. In rodents, hepatic CYP form induction can be associated with the formation of tumors by non-genotoxic modes of action in the liver, thyroid, and other tissues (Graham and Lake, 2008).

A major challenge in drug development is to accurately estimate human adverse drug effects to allow the selection and advancement of drug candidates with the best safety profile for further development. Due to species differences, safety data obtained with the routine *in vivo* studies with nonhuman laboratory animals do not always correctly predict human outcome. Human liver-derived systems, especially human hepatocytes, represent physiologically relevant experimental systems for the evaluation of human adverse drug effects (Li, 2011). The assays developed with human-based *in vitro* experimental systems for the assessment of two major adverse drug effects – drug-drug interactions and drug toxicity – can be used routinely during drug development to select and optimize drug candidates to enhance the probability of clinical success. These issues are now addressed in cryopreservation of human primary hepatocytes and the Integrated Discrete Multiple Organ Co-Culture (Li, 2008). A novel three-dimensional (3D) tissue-engineered scaffold system in a miniaturized setting has been developed, which can mimic multiple organ interaction in an "organ-on-chip" set-up. This is expected to revolutionize *in vitro* toxicology (Hosseinkhani et al., 2008). Along similar lines, a well-defined synthetic peptide that can self-assemble into three-dimensional interweaving nanofiber scaffolds to form a hydrogel, PuraMatrix, as a substrate for hepatocyte culture has been developed. Freshly isolated primary rat hepatocytes attached, migrated, and formed spheroids within 3 days after seeding on PuraMatrix, providing for three-dimensional hepatocyte culture, which answered the question of whether the *in vitro* system provides for the same cell-cell interaction as in an organ *in vivo* in its three dimensional architecture (Wang et al., 2008).

8 Alternative model organisms

In vitro approaches have several advantages but come with some limitations too. The *in vitro* test will not and cannot mimic the *in vivo* test one hundred per cent. At the same time, dealing with mammalian models *in vivo* raises ethical and moral issues. Thus, the possibility of conducting the tests *in vivo* in simpler organisms that belong to the lower levels of the phylogenetic tree and whose genome scale data are available is a viable option. There was a time when non-mammals were thought to be far from ideal materials for the study of biomedical sciences. However, it has now become abundantly clear that some non-mammals are not only convenient materials but also are endowed with physiological and pharmacological properties common to humans (Peterson et al., 2008). Because genes, receptors, and molecular processes are highly conserved across animal phyla, studies with other animal species could be representative for "higher," more complex animals (Hill et al., 2005). This approach is based on the level of sentience of the animal dealt with and also the capacity for self-renewal from ecological perspectives, combined with the availability in abundance, ease with which they can be raised and experimented upon, etc. To that effect, the zebra fish (*Danio rerio*), Drosophila (*Drosophila melanogaster*), the nematode worm *Caenorhabditis elegans*, and the simple cnidarian *Hydra* are now being proposed as *in vivo* alternative models that will be amenable to reduction and refinement alternatives.

The zebrafish, a small tropical fish native to the rivers of India and South Asia, has become one of the most popular model organisms in developmental genetics and (eco) toxicology. A number of unique features have contributed to its attraction, including its rapid development, easy maintenance in the laboratory, large number of offspring, transparency of embryos, and access to experimental manipulation. The genomic sequencing of zebrafish is almost complete and it is used as a model for human disease and development. Zebrafish embryos are now models for studying the effect of chemicals on gene and protein patterns, as well as the potential implications of differential expression for toxicity. These fish also are suitable for toxicokinetics, toxicodynamics, transcriptomics, toxicogenomics, proteomics, and metabolomics studies. The animal also offers biomarkers of endocrine disruption, immune modulation, genotoxicity, or chronic toxicity (Hill et al., 2005; Peterson et al., 2008; Scholz et al., 2008). Zebrafish can be

used to eliminate potentially unsafe compounds rapidly in the early stages of drug development and to prioritize compounds for further pre-clinical studies (McGrath and Li, 2008). The Vitellogenin1 mRNA induction assay has been proved to be a sensitive biomarker of exposure to organochlorine pesticides and brominated flame retardants (Chow et al., 2012). The zebrafish model has great potential for future applications in developmental toxicity testing in the context of REACH legislation, given that approximately 30,000 chemicals may need to be tested for safety, and under current guidelines such testing would require the use of approximately 7.2 million laboratory animals of which more than 80% would be used for examining reproductive and developmental toxicity (Lee et al., 2012). The zebrafish is a powerful whole animal model, complementary to *in vitro* and mammalian models in high-throughput behavioral screening of compound libraries (Ali et al., 2012). A recent report claims that zebrafish possess great promise in evaluating the toxicity of nanoparticles as well (Liu et al., 2012).

Drosophila has remained the most pioneering model organism for understanding concepts in genetics and developmental biology, including human diseases and toxicological research (Siddhique et al., 2005). Drosophila is the closest invertebrate model organism to humans, based on sequence similarity/conservation (reviewed in Tiwari et al., 2011). Its recently discovered application as a model organism in toxicology, especially in the context of NRC's vision of toxicology for the 21st century, has earned a new status for this fly; a new branch of toxicology, "Drosophotoxicology," has emerged (Rand, 2010). Its entire genome sequence has been analyzed, and its four chromosomes accommodate approximately 13,600 genes (Adams et al., 2000). Drosophila, with several distinctions in terms of biology and life cycle, provides great advantages for testing the variety of chemical toxicants to be investigated, the mode of delivery to the organism, the developmental stage, and the end points to be measured in determining biological/toxicological effects (Rand, 2010). Earlier, Drosophila served as an excellent *in vivo* model for genotoxic assessment (Siddhique et al., 2005). Drosophila fulfills most of the requirements of ECVAM, which recommended Drosophila as one of the promising organisms that will help answer complex questions in simple organisms (Tiwari et al., 2011).

Caenorhaditis elagans, the little nematode worm, has provided the discovery tools for several outstanding concepts in biology, particularly neuroscience, development, signal transduction, cell death, aging, and RNA interference, cell tracking, apoptosis, etc. (Antoshechkin Sternberg, 2007). The success of *C. elegans* as a model has attracted increased attention in the fields of biomedical and environmental toxicology. In fact, *C. elegans* has a number of features that make it not just relevant but quite powerful as a model for biological research. Its features have led to an increasing use of *C. elegans* in toxicology, both for mechanistic studies and for high-throughput screening approaches (Leung et al., 2008; Helmcke et al., 2010). In NRC's recommendation on alternatives to higher animal models, *C. elegans* occupies a promising place. It is an attractive model because of its well characterized and evolutionarily-conserved biology, low cost, and ability to be used in high-throughput screening (Boyd et al., 2010a, b). *C. elegans* is amenable to genetic manipulations such that it can be useful in unraveling mysteries in toxicology. For example, *C. elegans* expressing firefly luciferase has been developed, and it is useful in assessing effects of sub-lethal chronic exposure to environmental pollutants. Whole animal bio-luminescence is a valid toxicological endpoint and a rapid and sensitive predictor of toxic effects (Lagido et al., 2009). The advantages, such as conservation of disease and stress response pathways, availability of mutant and transgenic strains, and the wealth of biological information have led to increased use of *C. elegans* in toxicological studies (Boyd et al., 2012). Also, *C. elegans* contains cellular detoxification systems, including glutathione, metallothioneins, pumps and transporters, and heat shock proteins to regulate intracellular metal levels, and so provides several advantages for deciphering the mechanisms of metal detoxification (Martinez-Finley and Aschner, 2011). Most importantly, the toxic responses in *C. elegans* and mammalian toxicity were compared, and it was found that many endpoints were similar, indicating that measurements of morbidity and mortality in conjunction with morphology analysis in *C. elegans* may have the potential to predict mammalian toxic responses (Hunt et al., 2012). Thus, *C. elegans* represents an excellent complement to *in vitro* or cell culture-based systems and *in vivo* vertebrate models (Leung et al., 2008).

Hydra is a very simple eumetazoan diploblastic organism belonging to the phylum Cnidaria. Inhabiting freshwater bodies, this microscopic organism is by habit sedentary, attached to stones, pebbles, and plants. The cylindrical body has a basal disc with which it attaches to the substratum; at the opposite end is the mouth, located on top of a conical manubrium surrounded by 5-8 tentacles. The body has outer ectoderm/epidermis and inner endoderm/gastrodermis. Both layers are formed of musculo-epithelial cells but differ in finer details of structure as well as function. The primary method of reproduction is by budding, but it can reproduce sexually through development of transient gonads and the execution of fertilization between sperm and ova followed by the hatching out of a planula larva that undergoes encystment to overcome unfavorable conditions. *Hydra* has enormous powers of regeneration, and thus it has been a model organism for understanding regeneration, polarity, pattern formation, etc. This unique property is provided by the stem cells in *Hydra*, and the stem cell-ness renders *Hydra* an immortal animal. These aspects of simplicity have attracted the attention of biologists, and *Hydra* has since been a model organism in toxicity testing as well. *Hydra* spp has recently gained increased attention in aquatic toxicology as a sensitive and possible target species (Pascoe et al., 2003; Segner et al., 2003). Xenobiotic biotransformation, oxidative stress, growth, asexual reproduction, morphological changes, and feeding behavior could be conveniently determined in this organism. These endpoints represent key targets that underlie survival, growth, and reproduction, which form the basis of environmental risk assessment strategies (Quinn et al., 2012). In as much as morphology, regeneration, reproduction, feeding,

and attachment could be endpoints of convenience, biochemistry and bio-transformation of xenobiotics also are suitable targets to rely upon. *Hydra* is sensitive to metals, many organic toxicants, endocrine disrupting compounds, pharmaceuticals, nanomaterials, and industrial and municipal effluents. Drug/toxicant metabolizing enzymes have been identified in *Hydra* and are molecularly characterized (Dash et al., 2006, 2007). Above all, the toxicity endpoints now can be traced to the genes, since its full genome has been deciphered (Chapman et al., 2010). *Hydra* is so much a standardized lower animal model in aquatic toxicity testing that it was used in the WaterTox network, an international network of laboratories from eight participating countries that examined the applicability of a battery of simple, inexpensive bioassays in environmental management and the relevance of the test results in establishing the toxicological quality of water sources and drinking water (Dutka, 1989; Trottier et al., 1997). *Hydra* has become so popular in the arena of toxicological testing that it has staked a claim for appropriately designed, relatively simple, and inexpensive laboratory toxicity tests using it with a selection of acute and sub-lethal endpoints that are generally adequate, with small application factors, for predicting the environmental risk of polluting chemicals to freshwater ecosystems (Quinn et al., 2012)

9 *In silico* approaches

In silico toxicology is an approach to making precise and robust toxic predictions that can improve the risk assessment process while reducing the time span and economic burden accrued due to animal and/or *in vitro* testing. Hartung and Hoffman categorized *in silico* toxicology tools into 9 groups and identified QSARs as "the most prominent *in silico* tools at the moment" (Hartung and Hoffman, 2009). Quantitative Structure Activity Relationships (QSARs) is a mathematical modeling tool that has come into application to predict biological (toxic/pharmacological) activity associated with chemical structure. A set of parameters called descriptors is required to predict the toxic outcomes, such as genotoxicity, aquatic toxicity, and reproductive toxicity, to name a few. Different QSARs are employed depending on what type of toxic testing prediction is warranted. ebTrack, a new *in silico* tool, was developed as an integrated bioinformatics system for environmental research and analysis by addressing the issues of integration, curation, management, first level analysis, and interpretation of environmental and toxicological data from diverse sources. It is based on enhancements to the US FDA-developed ArrayTrack™ system through additional analysis modules for gene expression data, as well as through incorporation and linkages to modules for analysis of proteomic and metabolomic datasets that include tandem mass spectra (Chen et al., 2009).

ToxCast is another bio-informatics approach that was launched in 2007 by the US EPA and it is useful in developing ways to predict potential toxicity and to develop a cost-effective approach for prioritizing the thousands of chemicals that need toxicity testing. ToxCast helps explain how human body processes are affected by exposures to chemicals, and it helps determine which exposures are most likely to lead to adverse health effects. ToxCast testing methods include more than 650 high-throughput assays that can screen 2,000 environmental chemicals for potential toxicity. Phase I, Proof of Concept, was completed in 2009. It profiled more than 300 well-studied chemicals (primarily pesticides). These chemicals have more than 30 years' worth of existing toxicity data, since they have been tested already using traditional toxicology methods (primarily animal studies). Data from animal studies can be searched and queried using EPA's Toxicity Reference Database (ToxRefDB). Phase II currently is screening 1,000 chemicals from industrial and consumer products, food additives, and drugs[12].

The discovery of E-cell is expected to revolutionize the prediction of toxicity. The E-cell is a simulation package in which scientists modeled a hypothetical cell to carry out *in silico* experiments relating to protein-protein interactions, protein-DNA interactions, regulation of gene expression, and other features of cellular metabolism (Tomita et al., 1999; Takahashi et al., 2003). In as much as the potential of E-cell is being realized, further studies are being carried out to prove its applicability in the toxicological domain.

10 The EU Directives and legislation

Europe and the USA are forerunners and strong proponents of the alternatives concept. The EU Directive 86/609/EEC on "the protection of animals used for experimental and other scientific purposes" was amended and replaced by the Directive 2010/63/EU, which requires in Article 4: "*Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure*"; and in Article 47: "*The Commission and the Member States shall contribute to the development and validation of alternative approaches which could provide the same or higher levels of information as those obtained in procedures using animals, but which do not involve the use of animals or use fewer animals or which entail less painful procedures, and they shall take such other steps as they consider appropriate to encourage research in this field*."

In the realm of cosmetics, the Seventh Amendment to the EU Directive 76/768/EEC (the Cosmetics Directive) has become one of the most appreciated directions in favor of alternatives. It states that, as of 11th March 2009, Member States shall prohibit the marketing of cosmetic products containing ingredients or combinations of ingredients that have been the subject of animal testing using a method other than an alternative method. Three animal tests are exempt from this ban: repeated-dose toxicity, reproductive toxicity, and toxicokinetics, for which alternatives are not currently available and which can be used until a final cut-off date of March 2013, at which point they also will be banned.

[12] http://www.epa.gov/ncct/toxcast/

11 Agencies that validate alternatives

The European Centre for the Validation of Alternative Methods (ECVAM) was established in 1992 with the mandate to coordinate and promote the development and use of alternatives in basic and applied research and regulatory testing. It also helps promote dialogue among legislators, regulators, and all relevant stakeholders, particularly industry, biomedical scientists, consumer organizations, and animal-welfare groups, with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches. ECVAM has already validated fifty-five alternative methods that have reached the market, and more yet are in the pipeline[13].

Similarly, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was established in the USA in 1997 under the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) as an interagency committee of the US Government. ICCVAM coordinates interagency technical reviews of new and revised safety testing methods with regulatory applicability, including alternative test methods that may reduce, refine, or replace the use of animals in order to advance animal welfare while ensuring human health and safety[14]. The Japanese Center for Validation of Alternative Methods (JaCVAM) was established in Japan with a mission to promote the 3Rs in animal experiments for the evaluation of chemical substance safety in Japan and to establish guidelines for new alternative experimental methods through international collaboration[15]. The Republic of Korea's Centre for the Validation of Alternative Methods (KoCVAM)[16] is a recent addition to this list. International Cooperation on Alternative Test Methods (ICATM) was officially created in April 2009, when an agreement was signed between validation bodies from Europe, USA, Canada, Japan, and Korea[17] (Wind et al., 2010). A Brazilian Center for Validation of Alternative Methods (BraCVAM) is underway.

In light of the EU Directives, many companies are proactively choosing to eliminate animal and human clinical testing due to ethical considerations. Here, alternative methods are urgently needed to replace existing animal tests. It is very encouraging that the development of many promising *in vitro* methods to evaluate skin sensitization is moving forward steadily. ECVAM has initiated formal pre-validation of the human cell line activation test (h-CLAT), the myeloid U937 skin sensitization test (MUSST), and the direct peptide reactivity assay (DPRA). Each of these assays has entered the practical phase. KeratinoSense assay, a cell-based reporter gene assay, has forged ahead and soon will be subjected to ECVAM review. IIVS, a non-governmental organization based in Gaithersburg, MD, USA, is playing an important role in this endeavor.

The Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET), established in Germany in 1989, aims to bring about the replacement, particularly of legally prescribed animal experiments, with alternative test methods, to reduce the number of test animals to the absolutely necessary level, and to alleviate the pain and suffering of animals used in experiments[18].

12 REACH and its contribution to risk assessment

REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), EU, and ECHA (European Chemicals Agency) under it, aim to improve the protection of both human health and the environment while maintaining the competitiveness and enhancing the innovative capability of the EU chemicals industry. REACH makes industry responsible for assessing and managing the risks posed by chemicals and providing appropriate safety information to their users. According to REACH, as many as 30,000 single chemicals are required to register with a potential risk assessment requirement based on substance-specific data for the persistence, bioaccumulation, and toxic (PBT) properties for several thousand chemicals[19]. There are two major issues here: there are more than this number of single chemicals that would potentially affect human health, and given the number of tests to be conducted and the volume of data that needs to be generated, the target cannot be achieved anywhere near the deadline date. The situation becomes even more complex, since new chemicals will arise by the time evaluation of the existing chemicals is completed (Hartung and Rovida, 2009). From the perspective of "alternatives," this has introduced a challenge for industry, the regulatory agencies, and the toxicological research community to develop and validate alternative high-throughput testing strategies and limit the testing with animals by implementing the reduction, refinement, and replacement (the 3Rs) principle in ecotoxicological testing. In order to provide for safety evaluation data for this many chemicals – and perhaps more – the search is on for non-animal (alternative) testing methods for REACH (Lilienblum et al., 2008).

13 Non-governmental organizations and initiatives

In the field of basic biomedical research, the Center for Alternatives to Animal Testing (CAAT), established at Johns Hopkins University Bloomberg School of Public Health, U.S.A., aims to promote humane science by supporting the creation, development, validation, and use of alternatives to animals in research, product safety testing, and education.

13 http://ecvam.jrc.it/
14 http://iccvam.niehs.nih.gov/
15 http://jacvam.jp/en/
16 http://www.ksaae.org/
17 http://ihcp.jrc.ec.europa.eu/our_activities/alt-animal-testing/kocvam-joins-icatm
18 http://www.bfr.bund.de/en/zebet-58194.html
19 http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm

CAAT seeks to effect change by working with scientists in industry, government, and academia to find new ways to replace animals with non-animal methods, reduce the numbers of animals necessary, or refine methods to make them less painful or stressful to the animals involved[20]. The Center for Alternatives to Animal Testing, Europe (CAAT-EU) was founded on a collaboration between the Johns Hopkins Bloomberg School of Public Health and the University of Konstanz. CAAT-EU will coordinate transatlantic activities to promote humane science in research and education, and will participate, as partner or coordinator, in publicly and privately funded European projects[21].

Several European non-governmental programs are also helping steer the development of new approaches to toxicology. AXLR8, an EU project, seeks to accelerate the transition to a toxicity pathway based paradigm for chemical safety assessment by providing a forum for networking, information exchange, and collaboration[22]. The Risk Assessment in the 21st Century Project (Risk21), sponsored by the ILSI Health & Environmental Sciences Institute, part of the International Life Sciences Institute, is trying to look at the whole picture. In Risk21, approximately 90 scientists from industry, academia, government, and non-governmental organizations participate in four project teams that are focusing on ways to characterize real-world chemical exposure, incorporate dose-response information into risk assessment, develop a framework for toxicity testing, and account for the cumulative risk of exposure to multiple agents[23].

U.K.-based Unilever's skin sensitization program is developing *in vitro* and computational methods to assess the risk of contact dermatitis. To do this, the Unilever group is working to understand the biology of allergic responses in human skin and incorporating what they learn into computational models of the process[24].

The role that metabolomics has in the evaluation of xenobiotic toxicity studies is presented here, giving new ground to toxicology research. To provide a comprehensive assessment of this approach, the Consortium for Metabonomic Toxicology (COMET) has been formed by six pharmaceutical companies and the Imperial College of Science, Technology and Medicine (IC), London, U.K.[25]. The objective of this group is to define methodologies and to apply metabolomic data generated using NMR spectroscopy of urine and blood serum for preclinical toxicological screening of candidate drugs. This is being achieved by generating databases of results for a wide range of model toxins that serve as the raw material for computer-based expert systems for toxicity prediction. With the completion of 147 studies, the chief deliverables of a curated database of rodent biofluid NMR spectra and computer-based expert systems for the prediction of kidney or liver toxicity in rat and mouse based on the spectral data have been generated and delivered to the sponsoring companies. The project has met and exceeded all of its targets, and it was judged a resounding success by the sponsoring companies who are, in many cases, already enhancing and making use of the data in their in-house studies (Lindon et al., 2005).

Just as scientists raced to define the human genome, the Human Toxome Project (HTP) at Environmental Working Group is working to define the human toxome – the full scope of industrial pollution in humanity. HTP scientists use cutting edge biomonitoring techniques to test for industrial chemicals such as bisphenol A and perchlorate that enter the body through pollution or even as ingredients in everyday consumer products[26].

The Sens-it-iv project, aimed at novel testing strategies for *in vitro* assessment of allergens, has been launched to develop *in vitro* alternatives to animal tests currently used for the risk assessment of potential skin or lung sensitizers. The project participants include 28 groups overall, of which 9 represent industry, 15 represent universities or research institutes, and 4 represent organizations[27].

14 The NRC and toxicity testing in the 21st Century

Advances in molecular biology and toxicology are paving the way for major improvements in the evaluation of the hazards posed by the large number of chemicals found at low levels in the environment. The National Research Council was asked by the U.S. Environmental Protection Agency (US EPA) to review the state of the science and create a far-reaching vision for the future of toxicity testing. There was a landmark publication by the National Academy of Science (NAS) in June 2007, under the auspices of the National Research Council (NRC), *Toxicity Testing and Assessment in the Twenty-first Century: A Vision and a Strategy* (NRC, 2007). This report advocates sweeping changes in regulatory toxicity testing. It envisages a shift from current whole-animal based systems to testing founded primarily on *in vitro* methods, human cells in culture, *in silico* biokinetic modeling, and mechanisms of toxicity as understood through systems biology. The EPA, the National Toxicity Program (NTP), and the National Institutes of Health (NIH) signed a memorandum of understanding to develop and implement new high-throughput *in vitro* methods for testing chemicals and drugs. This will allow the collection of data requested in the NAS report. This significant step toward US development of alternative methodologies reflects both the best science and the most humane science.

20 http://caat.jhsph.edu/
21 http://cms.uni-konstanz.de/leist/caat-europe/
22 http://www.axlr8.com/
23 http://www.risk21.com/
24 http://www.unilever.com/sustainable-living/
25 http://bc-comet.sk.med.ic.ac.uk/
26 http://www.ewg.org/sites/humantoxome/
27 http://www.sens-it-iv.eu/

To help implement the recommendations of the NAS report as quickly as possible, the Doerenkamp-Zbinden Foundation (DZF) and CAAT collaborated to establish the *Transatlantic Think Tank of Toxicology*, or t^4. The t^4 is an effort, shared among CAAT at Johns Hopkins University, the University of Konstanz in Germany, and Utrecht University in the Netherlands, which seeks to advance evidence-based toxicology[28].

The Human Toxicology Project Consortium (HTPC) is a group of stakeholders currently drawn from the corporate and public interest communities who share the objective of accelerating implementation of *Toxicity Testing in the 21st Century*. The Consortium believes that the NRC vision is best implemented through a large-scale, international, coordinated effort similar to the Human Genome Project of the 1990s. This effort is called the Human Toxicology Project. The mission of HTPC is to serve as a catalyst for the prompt, global, and coordinated implementation of "21st century toxicology," which will safeguard human health and hasten the replacement of animal use in toxicology. Its vision is a global paradigm shift to an *in vitro* approach to the risk assessment of chemicals and drugs that is based on a modern understanding of human biology and disease pathways, yielding results more rapidly and more predictive of human health effects than current approaches. It includes Dow Chemical, DuPont, Exxon Mobil, The Hamner Institutes for Health Sciences, the HSUS family – Humane Society of the United States, Humane Society International, and Humane Society Legislative Fund, Johnson & Johnson, L'Oréal, Procter & Gamble, and Unilever as the partners. CAAT is an external partner. The key aims include technical (help promote the establishment and implementation of an international research roadmap), public policy (promote the development, adoption, and implementation of the new methodology through legislative appropriations and regulatory advocacy on a global basis), communication (promote greater appreciation of the need for a prompt and global transformation to the new paradigm among diverse stakeholders), and collaboration (build strategic partnerships to advance the vision of the Consortium, and, where appropriate, expand the Consortium)[29].

15 World and national congresses

Eight World Congresses on alternatives have been conducted[30], the latest being the "Eighth World Congress on Alternatives and Animal Use in the Life Sciences" organized at Montreal, Canada, on Aug 21-25, 2011[31]. National Congresses have been conducted in several countries, including India[32]. These achievements were made possible by the aggressive campaigning of and benevolent contribution from philanthropic organizations such as Fund for the Replacement of Animals in Medical Experiments (FRAME), UK[33], Doerenkamp-Zbinden Foundation (DZF), Switzerland[34], 3R Foundation, Switzerland[35], Humane Society of the United States (HSUS)[36], American Anti-Vivisection Society (AAVS)[37], International Centre for Alternatives in Research and Education (I-CARE)[38], People for Ethical Treatment of Animals (PETA)[39], etc., which fund hundreds of projects pertaining to the innovation of new testing methods based on the 3Rs principle.

16 Scientific journals on alternatives

Scientific journals specifically dealing with animal alternatives include *Alternatives to Animal Testing and Experimentation* (AATEX), *Japanese Society of Alternatives to Animal Experimentation* (JSAAE), *Alternatives to Laboratory Animals* (ATLA), *Alternatives to Animal Experimentation* (ALTEX), *In Vitro Cellular and Developmental Biology – Animal, and Toxicology In Vitro*.

17 Conclusion

The 3Rs have steadily become more widely known and better appreciated, although much still has to be done to ensure their broader implementation. The discussion of issues such as unnecessary use or reasonable alternatives and their interpretation according to the law is essential for a critical, workable, and consensual approach to the 3Rs principle. At the same time, pertinent and well-designed alternative experiments, political support, financial assistance and technical acumen are to be brought in for the development and advancement of the alternatives dogma. More important is for those countries that are lagging behind to catch up and realize that "alternatives" is not just humane science but better science. It is also time for India to catch up with the international trend and for the regulatory authorities to approve adoption of the validated alternative methods in drug testing and toxicology.

28 http://altweb.jhsph.edu/about_us/t4.html
29 http://htpconsortium.wordpress.com/
30 http://alttox.org/spotlight/049.html
31 http://www.wc8.ccac.ca/pages/welcome/
32 http://www.icare-worldwide.org/indian_congress/
33 http://www.frame.org.uk/
34 http://www.doerenkamp.ch/en/
35 http://www.forschung3r.ch/index_en.html
36 http://www.humanesociety.org/
37 http://www.aavs.org/
38 http://www.icare-worldwide.org/
39 http://www.peta.org/

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