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Required Effort to Evolve Toxicity Testing Using *In Vitro* Methods

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Introduction

The procedures used to conduct chemical safety evaluations have developed over the years and continue to evolve with our understanding of the science of toxicology. The main objective of toxicity testing is to generate a toxicological database which can be utilized in human safety evaluation. Historically, the database to be utilized in the safety evaluation process was developed using whole-animal testing, human epidemiological studies and, in some cases, accidental human exposure data. However, as a result of recent biotechnological advances in the areas of cell culture and bioanalytical methodologies, new possibilities for *in vitro* studies and their applications to toxicity testing have been created. In light of these developments, the traditional approach to toxicity testing should be reevaluated.

What are the advantages and significant problems of whole animal testing?

Whole-animal testing has certain advantages which have made these methods attractive over the years. First and foremost, a whole-animal model for toxicity testing provides as integrated biological system which serves as a surrogate model for the complexities of human beings. This variety of interacting biochemical and physiological systems offers a wide net in which to catch potential toxicological responses. Second, whole-animal systems provide information about specific target organs for toxic chemicals, as well are useful in understanding toxicodynamics, such as the processes of absorption, distribution, metabolism and excretion of toxicants, which is essential for risk assessment. Finally, whole-animal systems is suitable for measuring the chronic effects of chemicals. Thus, whole-animal toxicity testing has several strengths which support is continued use.

However, there are also significant problems in using whole animals which must be recognized. First, the issue of significant public concerns with respect to animal welfare. Second, whole-animal testing is expensive in terms of the time and cost involved in generating the complete databases needed for risk assessment. A third major problem involves the extrapolation of the available animal toxicological data to humans. Inter-species extrapolation addresses the issues involved in making data obtained from animal models relevant to man.

What are the advantages of *in vitro* testing systems?

What advantages, if any, do *in vitro* testing systems have to offer? First, *in vitro* testing has the potential to be more rigorously standardized than *in vivo* testing. This has important advantages since reliable, quality controlled data can be generated. Eurthermore, *in vitro* systems are generally faster and less expensive, thus offering an economic advan-

tage. Also, because human cells can be used directly *in vitro* test systems, the question of species differences can be eliminated. In addition, *in vitro* systems offer good experimental control of the cellular dose of chemicals. This is an important factor in obtaining dose-response relationship databases. Finally, *in vitro* toxicity testing offers the advantage of reducing the use of live animals and the quantities of test chemicals in toxicity evaluation studies, which are important from a societal point of view.

What is the current status of *in vitro* initiatives in toxicity?

What is the current status of *in vitro* initiatives in toxicity testing? Some important categories of research include: cytotoxicity, irritation and inflammation, genotoxicity, teratogenicity, target organ toxicity, toxicokinetics and structure-activity relationships. This is not an inclusive listing, but it covers the most general categories in which *in vitro* approaches are actively being investigated.

In vitro cytotoxicity testing assays are designed to evaluate the intrinsic ability of a chemical to kill cells. Many *in vitro* cytotoxicity assays have been developed over the years. Some were developed for special purposes, such as screening potential anti-neoplastic drugs for their ability to kill cancer cells, while others were developed for more general purposes. Cytotoxicity can be evaluated with any cell type that can be cultured *in vitro* and methods for evaluating whether or not cells are dead have multiplied rapidly in recent years. Four cytotoxicity test systems which have received particular attention in the toxicity testing area are the cell growth measurement assay systems that measure colony formation ability (Watanabe et al., 1989), total cell protein, neutral red uptake assay (Torishima et al., 1991), and the dehydrogenase enzyme assay (Ishiyama et al., 1995). The main advantage of these tests is that they can be automated so that many chemicals can be rapidly tested at relatively low costs. A recent review of the use of the Draize test and potential alternative testing methods identified that a large number (34 methods) of *in vitro* tests, including cytotoxicity testing assays, existed at that time which could potentially play a role in a test battery to replace the irritation and inflammation testing.

We developed colony assay system as an alternative to the Draize testing and determined the cytotoxicity effect of 52 chemicals using cosmetic products on primary rabbit cornea cells. A dose-(exposure-) response curve can be generated for each chemical, and the concentration that produces 50% inhibition of colony formation (LD_{50}) is determined. This LD_{50} value can be compared to the LD_{50} for known chemical toxins to obtain an evaluation of the relative cytotoxicity of a new chemical. A significant correlation between the relative toxicity in rabbit cornea cells *in vitro* and relative eye irritation in the Draize test was seen with treatment with 52 chemicals used in consumer products ($r = 0.91$).

In the field of genotoxicity, many approaches, beginning with the Ames bacterial assay, have been proposed and are under development. This area has probably been the most adequately funded and active area of *in vitro* approaches to toxicity testing as a consequence of the fact tht a whole animal test for carcinogenicity is extremely expensive and time-consuming. Therefore, there is significant economic pressure to develop alternatives to *in vivo* carcinogenicity tests using mammalian cells—especially human cells, which should be developed in future.

The *in vitro* transformation system using Syrian hamster embryo cells is useful for detecting the carcinogenic potential of radiation and chemicals (Watabane et al., 1990, 1991). Although morphological transformation is the first observable and tentative change in cells soon after carcinogen-treatment, the only progeny of them express

other transforming phenotypes during extensive subculturing, and are able to produce tumors in animals (Watanabe and Suzuki, 1991). This assay system has a high predictive value for the detection of cardinogens, including a number of non-mutagenic carcinogens.

In contrast to general cytotoxicity, target organ toxicity is imoprtant in understanding the subchronic and chronic effects of chemicals *in vivo*. Extensive progress has been made in the use of *in vitro* testing for heart, kidney, liver, lung and nervous system toxicity. The key to target organ testing is to establish the cell cultures from specific organs with differentiated phenotypes. Data from this research is also helping to solve the problems of inter-species extrapolation of testing data.

Toxico-kinetics must receive high priority in future research, because of the importance of extrapolating from *in vitro* to *in vivo* (Kotani, et al., 1994).

Other important research areas are teratogenecity and structure-activity relationships (Sugai et al., 1990). Thus, research activities of *in vitro* testing have recently accelerated in many field and have many advantages, as mentioned above.

What is delaying the replacement of *in vitro* methods?

What is delaying the replacement of *in vivo* methods by *in vitro* toxicity testing systems? The major limitation is that *in vitro* toxicity testing methodology is not yet fully accepted by either scientific or regulatory communities. The historical database needed to fully define the limitations of these systems does not yet exist. In should be emphasized that there is no one *in vitro* test which is going to answer all toxicological questions. The complexity of whole animals will require a battery of *in vitro* testing. In the best case it will take a battery of several *in vitro* tests to obtain the necessary information to evaluate specific human risks resulting from exposure to toxic chemicals. Significant scientific and technical problems must be overcome in the development of *in vitro* toxicity testing; however, these methodologies are viewed as a new direction for the science of toxicity testing at present, and in the future. In should be obvious from these examples that *in vitro* toxicity testing is a new and developing field of toxicology. Its ultimate success will depend on scientific breakthroughs in various areas of toxicology and cell culture.

A major goal of *in vitro* testing is to use human cells for all testing systems to eliminate species extrapolation questions. To attain this goal, several technological obstacles must be surmounted. First, not all human cells can be adequately cultured. Those that can, suffer from the phenomenon of differentiation, where the cells take on the characteristics of more primitive cells than the normal cells found *in situ*. Significant research activities are in progress to maintain differentiated cells in culture, but more research is needed in this area. Secondly, the supply of normal human cells for toxicological testing activities is limited. Biotechnology and genetic engineering must solve this supply problem to make human cells a commonly available resource. Finally, the problems of *in vitro* to *in vivo* extrapolation must be overcome. The extrapolation from tissue culture to man can be expected to be a difficult problem. However, with a focused and well supported research effort, the problems can be defined and extrapolation procedures established.

Things sought in the development of alternative research

The animal protection movement has forwarded on "Alternative Research for Animal Experimentation" during the last 10 years. Therefore, many people believe that the trend of "Alternative Research" has started to cope with this and have judged that such studies are unproductive. However, I think that the original meaning of "Alternative Research" in toxicology is the technological development of

making new analytical systems for life science. Judging from this meaning, "Alternative Research" is advancing in a pleasing direction. Newly developed technology should be widely spreaded. In this meaning, technology transfer system have to established. Also, education about alternative research is the most important for development of alternative research.

We have recently heard the phases "kindness to humans" and/or "kindness to the earth" from TV commercials very often. What is "kindness"? These phrases point to environmental problems. In this meaning, I think that the development of new analytical methods to detect environmental pollution and to measure the toxicity of chemicals is the science that produces "kindness for lives and earth". Although it doesn't produce a direct profit, it should produce a new general idea about "kindness". Scientists, corporations and administrative officers should bear the responsibility for this.

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