VII F. Advances in the Development of Alternatives to Whole Animal (Vertebrate) Testing

Because animal experimentation has become an emotional issue, it is important to recognize the growing impact of *in vitro* toxicology on the practice of toxicology. Although the field is often termed "alternative," experimental models have been applied to the three "R's" of Russel and Burch: [1] to replace animal models, to reduce the number of animals used, or to refine test methods to minimize stress and suffering to animals.

This section is not intended as a guideline but serves to identify a future direction in methodology. In the context of this document, "alternatives to whole animal (vertebrate) experimentation" refers to *in vitro* tests for potential toxicity that substitute for or replace *in vivo* (whole animal) studies. "*In Vitro*" literally means "in glass", and is interpreted to mean "in a test tube" or "outside of the body".[2] Alternative tests include short-term tests using isolated cells, tissues, and organs and studies involving mathematical modeling, epidemiology, or the use of human volunteers; short-term tests for genetic toxicity (see **Chapter IV C 1**) are excluded.

In practice, alternative tests are used to support the planning and interpretation of whole animal toxicity studies and are not yet used as substitutes for toxicity studies using whole animals. For example, an alternative test may be used 1) to determine the relative biological potency of a series of toxicants at the cellular level, 2) to select the animal model in which to conduct an *in vivo* test by comparing the metabolic properties of a toxicant at the cellular level in several species, and 3) to identify mechanism(s) of toxicity by defining the relationship between exposure to a toxicant and development of various toxicological endpoints at the cellular, subcellular and molecular levels of organization.

Recent advances that have been made in *in vitro* studies with isolated cells, tissues, and organs have directed the scientific community toward developing, validating, and evaluating alternative test systems. The predictive value of a standardized test must be assessed by means of a series of validation studies. Validation can demonstrate that the use of an *in vitro* test is equivalent to the use of an established *in vivo* test or that the *in vitro* test accurately predicts human toxicity. Anticipating a continued increase in the development and use of alternative *in vitro* test systems, [3,4] the Agency encourages the development of approaches that can provide information relevant to the assessment of human risks.

1. Reasons for Developing Alternative Tests

Several reasons to encourage the development of alternative *in vitro* tests are listed below:

- Economy and efficiency: Once established, *in vitro* tests may provide toxicity information in a cost-effective and time-saving manner. Information generated from *in vitro* test systems can be used to increase the efficiency of whole-animal studies and decrease the number of animals used in toxicity testing. The relative simplicity and space-saving characteristics of *in vitro* methods also are viewed as advantages.

- Information about human risk: Human cells, ethically obtained and successfully established *in vitro*, may provide information about a toxicant that is relevant to human risk. For example, a toxicant's mechanism of action or metabolism in human cells can provide the basis for selecting a suitable animal model for long-term toxicity studies.

2. Possible Applications of Alternative Tests

- Isolated cells, tissues, and organs can be prepared and maintained in culture by methods that preserve

properties characteristic of the same cells, tissues, and organs *in vivo*. Using such *in vitro* systems will permit data to be generated under controlled experimental conditions and in the absence of many complicating factors characteristic of experiments with whole animals. For example, the use of cell culture systems will enable the metabolism of a toxicant that occurs in one type of cell (*i.e.*, hepatocyte cells) to be studied separately from a toxic endpoint that occurs in a different cell type.

- Several toxic endpoints may lend themselves to quantification in an *in vitro* test system. Relevant endpoints could be identified by comparing the action of a toxicant at cellular, subcellular or molecular sites with the toxic effects observed in the target organ or tissue *in vivo*. Analysis of a broad spectrum of *in vitro* cellular events may provide information about the *in vivo* progression of a toxic response as a function of toxicant concentration and time.

- Because *in vitro* procedures have the potential to yield reproducible measurements, they theoretically lend themselves to standardization. However, interpreting data obtained from a standardized *in vitro* toxicity test with a reasonable degree of confidence can only occur after potential confounding factors, such as interactions between the test agent and non-cellular components of the test system, have been identified or eliminated. [5]

- The process of validation appears to be key to the full acceptance of alternative tests where the reliability and relevance of procedures are established for specific purposes.[6] While there is much discussion about the framework for this process, several components appear essential to the overall coordination of the validation process, including: scientific consensus on the definition of a validated test, reference chemicals with defined toxicity and general availability, a central repository for test performance data and protocols, an established network of laboratories with the capabilities of method validation, and scientific understanding of the mechanistic basis of the toxicological process involved. An impartial and competent group of scientists from regulatory agencies and the research community could facilitate the implementation of the validation process.

3. Limitations of Alternative Tests

Limitations of *in vitro* tests are well known. For example:

- *In Vitro* test systems are not available for all tissues and organs. In addition, normal systemic mechanisms of absorption, penetration, distribution, and excretion are absent from *in vitro* test systems. *In Vitro* systems lack the complex, interactive effects of the immune, blood, endocrine systems, nervous system, and other integrated elements of the whole animal. Thus, *in vitro* tests cannot be used to study the complex nature of systemic toxicity.

- Validation of new methods is time-consuming and expensive; acceptance of *in vitro* tests as alternatives to traditional toxicity testing in whole animals is expected to be slow. [7] While many schemes have been proposed to expedite these processes, no alternative *in vitro* test presently can replace an *in vivo* toxicity study.

4. Current Use of *In Vitro* Tests

Numerous & diverse *in vitro* tests have been developed. Their importance and use have been discussed in any publications. [8-23] Many of these tests will be improved over time by the introduction of new scientific information and technological advances in *in vitro* toxicology and related fields, such as molecular biology and biotechnology. The Agency encourages the development and use of *in vitro* test systems for planning and interpreting the results from whole animal toxicity studies.

Significant advances have been made in the development of *in vitro* alternatives for ocular safety testing.[24-27] Other *in vitro* systems have been proposed which measure a broad range of endpoints and are now in various stages of validation. The Agency is currently part of an interagency regulatory groups evaluating these proposed alternative test methods.

In Vitro approaches to toxicity testing can provide useful data when integrated with other information about the toxicity of food and color additives used in food. Results of *in vitro* tests can be used to optimize the design of conventional toxicity tests for a particular test substance by helping to determine appropriate dose levels and by helping to decide which species is the best model for man. Such improvements in the design of whole animal toxicity tests may reduce the number of test animals required to produce useful information about the safety of proposed food and color additives used in food.

In Vitro tests can help elucidate the nature of the interaction between test substance and organism at the cellular, subcellular, and molecular levels. Thus, once the critical target organ or organ system has been identified in whole animal studies, *in vitro* tests can focus on the mechanism of action of the test substance at the target site. Information from these studies can assist the Agency in making decisions about the safety of proposed food and color additives used in food by comparing responses observed in human and animal cells and by facilitating extrapolation from high-dose to low-dose responses.

At present, in evaluating a petition for the use of a food or color additive, the Agency considers *in vitro* tests to be useful in helping to identify the mechanism(s) of action of the test substance and to provide information about subtle effects observed *in vitro* that may not be observed in *in vivo* studies

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