

## ANNEX

# *General Aspects of Test Procedures in Reproduction Toxicology\**

### INTRODUCTION

The response of a biological system to a chemical stimulus is related to the toxicity of the test material, the dose level, the duration of exposure and the route of administration. Reproduction toxicology is particularly concerned with developmental stages (embryonic, fetal, lactational and pubertal) and gonadal cyclic or maturational activities. The absorption, distribution, biotransformation and elimination of administered substances must be taken into account when interpreting observed effects. Different sections of this annex must be considered, therefore, within the framework of all the available toxicological knowledge developed in relation to any test material. In addition, uniformly stringent test requirements are necessary for all chemicals. An equally important premise in developing cost-effective information is that the effort expended on testing a chemical for safety have some semblance to the true upper limit of the potential for harm of a given compound. This concept has been referred to as the 'principle of commensurate effort' and has also been used as a means of making testing requirements more cost effective.

An important consideration in the selection of appropriate reproduction tests is the intended use of the results. In general, test data are generated for three major purposes:

- (1) new product screening;
- (2) regulatory approval of new products; and
- (3) safety review of chemicals already used and present in the human environment.

The many types of products to be tested include food additives, agricultural chemicals, pesticides, pharmaceuticals, cosmetics, environmental contaminants and industrial chemicals. Sometimes the intended use of the chemical also influences the selection of tests. Thus, the selection of tests in a given test programme depends on exposure, observed toxicity, purpose of testing and the intended use of the chemical.

\* Extract from the report of the Workgroup on female reproductive function of mammals.

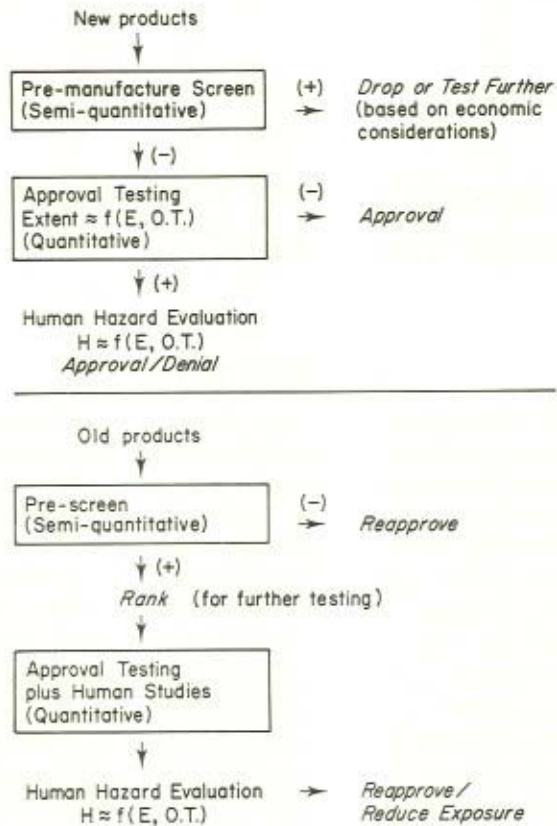


Figure 1A Testing of new and old products E, exposure level; O.T., observed toxicity; H, human hazard

### TEST VALIDATION

In order that a reproduction toxicology test be selected for use in an evaluation programme, it should be valid for the assessment of risk to reproduction by meeting the following criteria.

- (1) It should be reproducible in a single species.
- (2) The results in one species of experimental animals should be reproducible and predictive of results in one or more additional species of experimental animals.
- (3) The results in experimental animals should be predictive of a human effect.
- (4) The results in experimental animals should allow quantitative assessment of risk to human reproductive function.

The first criterion is simple to assess through repeated testing and interlaboratory comparisons. The second is more difficult to achieve in reproduction studies because of interspecies differences in reproduction systems (even among mammals) and in absorption, distribution, biotransformation and excretion of different compounds.

Even more difficult, and often impossible, is the validation of a test for human subjects. Direct epidemiological studies of effects on reproduction often cannot be carried out because human exposure has not occurred and cannot be achieved experimentally. Even when exposure has occurred, estimates of exposure dose are usually very difficult or impossible to make.

A possible approach to this problem would be to develop an understanding of the effects of chemical agent(s) at the cellular level and to demonstrate similar effects in human biopsied or cultured materials. This would be subject to the qualification that variations may occur in absorption or metabolism of the substance before it acts at the cellular level.

The demonstration that a test performed on an animal model system is predictive of human effects in a number of situations, where this is testable, would provide the best possible qualitative validation of any test to be used for human risk assessment.

The most important criterion required for quantitative validation of any test to be used for risk assessment is that the positive test result shows dose response. Cause-effect is most clearly established in situations where response (the number of individuals in an exposed population showing the effect) is positively correlated with dose. Because estimation of dose is difficult to accomplish in human populations, there are very few reliably determined human dose-response relationships for chemicals.

## THE CHOICE OF TESTS

The choice of testing procedures depends on the purpose of testing. The most common purposes are outlined below.

### **Pre-manufacture Screening of New Products**

The characteristics of the tests that are selected for this type of screening are in general as follows:

- (1) end-points are semi-quantitative;
- (2) the test should be convenient for screening large numbers of compounds;
- (3) the tests should predict the biological hazard potential;
- (4) tests should be rapid, cost effective and reproducible;
- (5) negative responses in a test should indicate a low probability of effects occurring for the end-point measured;



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- (6) the test battery should cover as many reproduction variables as possible;
- (7) test data are generated for 'in house' evaluation;
- (8) human exposure associated with testing should be minimal; and
- (9) metabolic considerations should be included.

### **Testing of New Products for Regulatory Approval**

Characteristics of tests selected for this purpose are as follows:

- (1) tests must be quantitative and it should be possible to extrapolate the results to human subjects;
- (2) test designs must cover the anticipated exposure as well as a wide range of other exposure levels;
- (3) where possible, routes of exposure should reflect anticipated or actual human exposure patterns;
- (4) tests should be cost effective and reproducible; and
- (5) test designs and data analyses must include metabolic considerations.

### **Safety Review of Chemicals in the Environment**

Characteristics of tests selected for this purpose include the following:

- (1) rapid or short-term screening procedures should be such that testing results can be used in priority ranking; and
- (2) human studies can also be included for these compounds. The types of human studies may involve identification of populations at risk, followed by retrospective or prospective epidemiological studies and clinical monitoring of identified populations.

## **EXAMPLES OF TESTS ASSOCIATED WITH THE IDENTIFIED TEST AREAS**

### **Proposed New Product Screens**

These tests should be primarily short-term, inexpensive, and should have at least semi-quantitative reproductive function end-points. Examples are:

- (1) fertility tests (see section 2.1.2);
- (2) preimplantation development (see section 2.3.1);
- (3) *in vitro* test for embryotoxicity (see section 2.3.3.2); and
- (4) possible non-mammalian test systems (future development).

### **Approval of New Substances**

These tests are discussed in section 2.1 on integrated reproduction function studies, including:

- (1) multigeneration studies;
- (2) fertility studies;
- (3) *in vivo* tests for selective embryotoxicity; and
- (4) peri- and postnatal studies (see sections 2.3.3.4 on parturition and 2.3.3.5 on lactation).

### **Safety Review of Chemicals Already Present in the Human Environment**

Tests for this purpose involve both screening procedures and regulatory tests described above as well as screening of effects on human reproductive function and epidemiological studies of exposed populations.

