

The Assessment of Mutagenicity

Health Protection Branch
Mutagenicity Guidelines

Environmental Health Directorate
Health Protection Branch

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Summary

The Health Protection Branch (HPB) Executive Committee established the Branch Genotoxicity Committee (BGC) to develop recommendations on a Branch-wide approach to the use of genotoxicity test results in toxicological assessment. This report, endorsed by the HPB Executive Committee, addresses the mandate under seven headings.

I. Introduction

The needs of the three Directorates (Drugs, Environmental Health, and Food) concerned in chemical regulation are similar insofar as each is called on to regulate large numbers of chemicals. The pioneering effort of the Environmental Contaminants Advisory Committee on Mutagenesis is acknowledged as their report suggests a rational answer to the problems presently confounding the value of genotoxicity tests for chemical and product toxicology assessment. That Committee recommended the definition and use of "Levels of Concern" (LOC) to rationalize a systematic approach to testing.

It was noted that the weight given to various genetic toxicology tests, and what was considered to be adequately conducted and reported tests, differed considerably, not only among Directorates but also among individuals within the same Directorate. Consequently, there is a need for much improved data evaluation guidelines in this area.

II. Genotoxicity Tests

A large number of genotoxicity tests are presently available for use in hazard evaluation. These tests detect the two main categories of mutations, gene mutation and chromosomal aberration, as well as indications of DNA damage. Tests to assess these endpoints can be carried out both *in vitro* and *in vivo*, with *in vivo* tests being conducted in germ cells, as well as in somatic cells. In order to assess adequately any expression of genotoxicity, a simplified systematic approach to the selection of these tests is required.

Therefore, there must be an ordered approach using a limited number of well-defined tests that complement each other in terms of endpoints, and that permits a systematic assessment of genotoxicity.

III. Mutagenicity Testing Strategy

A three level approach to genotoxicity testing based on the Level of Concern strategy in the report of the Environmental Contaminants Advisory Committee on Mutagenesis has been adopted by the HPB.

Chemicals for which human exposure is high or widespread merit the greatest initial concern (LOC III), while those to which few people are exposed at very low dose levels would be of least concern (LOC I). An intermediate level (LOC II) is also defined. Data from at least two different short-term *in vitro* tests would be standard for the toxicological evaluation of all chemicals for which human exposure is involved. Accordingly, substances assigned initially to LOC I should be subject to two short-term tests, one for gene mutations and the other for chromosomal aberrations *in vitro*. Chemicals initially assigned to the higher levels of concern (LOC II and III) should be subject to similar scrutiny, but additional data should be obtained initially for chromosomal aberrations in mammalian cells *in vivo*. The proposed scheme stresses the importance of confirming potential effects found *in vitro* by performing the appropriate tests in animal models, rather than attempting to estimate hazard by merely tabulating the number of positive and negative *in vitro* and *in vivo* test results. The appropriate type of regulatory action to be taken is suggested for different outcomes. Accordingly:

- 1) Directorates with a mandate to regulate the use of chemicals should adopt the allocations of these chemicals to the specific Level(s) of Concern;
- 2) the interpretation of test data and the subsequent action should be based on the strategy indicated for that Level of Concern.

IV. Genotoxicity Tests as Predictors for Carcinogenicity

It is evident that the earlier optimism that *in vitro* short-term tests would eventually replace the rodent bioassay was unfounded. There are two major sources of discordance between genotoxicity assays and the cancer bioassay. First, mutagenicity tests will not detect agents that induce cancer by non-genetic mechanisms. Second, *in vitro* genotoxicity tests have a much higher level of sensitivity than *in vivo* tests including the cancer bioassay and thus, tend to overpredict *in vivo* activity. The accuracy of these tests is reviewed and

discussion is presented on the pharmacokinetic considerations of both *in vitro* and *in vivo* testing.

V. Germ Cell and Somatic Cell Mutation

Because most recognized human germ cell (inherited) mutation is usually expressed early in life, it is the cause of major demands on medical and social services and is often the cause of protracted human suffering. There is, however, relatively little known about the agents responsible for human germ cell mutation. The reasons why human germ cell mutations have not yet been definitely attributable to specific agents are considered. It was recognized that a significant proportion of agents that cause *in vivo* somatic cell mutation might also possess the ability to lead to mutation in germ cells that may be transmitted to offspring. When evidence for *in vivo* somatic genotoxicity is demonstrated along with tissue distribution, metabolic and/or pathologic evidence that the genotoxic chemical (or metabolites) reaches the germ line (whether or not overt effects on fertility are found) the possibility of induced genetic damage to germ cells leading to heritable effects should be evaluated.

While models for risk extrapolation of heritable mutations in animals to human beings are available, they lack sophistication because of assumptions that are currently utilized. Further research-based effort should be directed toward establishing the scientific links necessary to reduce the assumptions required in risk estimation for heritable effects.

VI. Validation of Genotoxicity Tests

The ability of genotoxicity tests to detect and predict effects that are recognized as being detrimental to human health is discussed. The process of test validation has been subdivided into two concepts depending on the relationship of the test to the human health effect with which it is associated. The first concept examines the relationship between the measured and the associated health effect. In this case, the genotoxic endpoints are considered in their relationship to a health effect, such as cancer. In the second concept, the endpoints measured and the health effect observed are directly related. An example of this concept would be the relationship between the rodent bioassay and the development of tumours in humans. Here, assumptions from species extrapolation become important considerations.

VII. Epidemiological Extension of the Regulatory Process

The potential for human somatic cell genotoxicity to strengthen current efforts to prevent the exposure of human populations to genotoxic agents was recognized. Some *in vivo* tests could be used non-invasively in epidemiological studies on selected human populations to determine directly whether human genotoxicants had evaded the assessment process and what, if any, adverse health effects had resulted.