Short-term Toxicity Tests for Non-genotoxic Effects
Edited by P. Bourdeau *et al.*

CHAPTER 2

*Non-specialized Mammalian Cell Cultures for Toxicity Testing**

2.1 *IN VITRO* METHODS FOR THE ASSESSMENT OF GENERAL CELLULAR TOXICITY

These tests aim at detecting changes in common basic functions, processes and the structure of cells. These types of test have been referred to as 'general cell toxicology investigations' (Paganuzzi-Stammati *et al.*, 1981) or 'basal cytotoxicity tests' (Ekwall, 1983). Typically, they utilize cultures of undifferentiated cells (i.e. cells showing no organ-specific characteristics). These cell systems are relatively easy to culture and do not change from test to test (Ekwall, 1983). They include fibroblastic and epitheloid cell systems such as diploid human fibroblast lines and HeLa cell cultures.

Ekwall *et al.* (Chapter 7, this volume) point out that basic toxicity has often been studied in elaborate organ-specific culture systems leading to difficulties in interpretation because of confounding basic and cell-specific effects.

2.2 END-POINTS

Numerous end-points have been used by different investigators to measure toxicity. These include growth determined by protein analysis, plating efficiency, enzyme release, exclusion or inclusion of dyes or radioactive markers and metabolic alterations such as oxygen consumption and ATP levels. Most of these end-points are quantitative and can be used to plot dose–response curves. This should facilitate interlaboratory comparisons and the application of quality control methods to ensure the reliability of the results obtained. The cytotoxic concentrations of chemicals determined *in vitro* have been shown to correlate well with lethal doses in laboratory animals and man for a range of selected drugs and chemicals (Ekwall, 1983).

Morphological changes in cells exposed to chemicals, observed by light or electron microscopy, have also been used to demonstrate basic cytotoxicity. Effects

*This chapter was prepared by a Workgroup chaired by A. N. Rowan. Other members were A. Berlin (rapporteur), G. C. Becking, B. Ekwall, N. Fernicola, J. Friedrich, M. I. Gounar, F. Kaloyanova, C. R. Krishna Murti, B. Ordonez, I. V. Sanockij, and A. L. Stammati.

commonly observed include cytoplasmic blobs suggesting injury of the cell membrane and vacuolization (Ekwall, 1983). Although qualitative in nature, these observations may provide valuable information about the pathologic processes that occur as a consequence of exposure to a chemical substance.

2.3 CURRENT LIMITATIONS AND THE POTENTIAL FOR IMPROVED METHODS

These tests have the merit of being rapid and inexpensive to perform although a relatively high degree of skill is required to obtain reliable results. However, culture systems and conditions currently employed vary considerably from one laboratory to another. Lack of standardization in procedures has hindered inter-laboratory comparison of results that are germane to their proper validation as adequate test methods. At the present time, these tests have mainly found application as adjuncts to *in vivo* investigations, or as a screening technique.

Because basic cytotoxicity tests evaluate only the effects of a chemical on basic cellular processes, these tests have limitations in their value for predicting *in vivo* toxicity. These limitations include:

(1) Basic cytotoxicity tests do not evaluate the capacity of highly specialized cells to carry out their organ-specific functions. Organ-specific toxicity requires testing *in vivo* or in suitable differentiated cell cultures.
(2) Some types of toxicity involve the interactive influence of different types of cell. Toxic responses involving hormonal and nervous adjustments and immunological responses, for example, are typical of those involving organizational features characteristic of the whole organism. This type of response requires *in vivo* testing.
(3) Many chemicals require prior biotransformation before they exert a toxic effect on cells. In such cases, tests with hepatocytes or other metabolically competent cells could be used to supplement tests on less competent cells. However, it should be noted that hepatocyte cultures undergo dedifferentiation and, as a consequence, may modify their capacity to metabolize xenobiotic chemicals (Paganuzzi-Stammati *et al.*, 1981).
(4) *In vitro* concentrations of substances may be difficult to relate to concentrations in intact animals because of distribution phenomena (e.g. blood–brain barrier).
(5) Most tissue culture systems involve such short incubation and observation times that they may be predictive only for acute *in vivo* effects. It is doubtful that such tests can be used to predict chronic toxicity.

2.4 CONCLUSIONS AND RECOMMENDATIONS

(1) A number of *in vitro* methods to measure general cytoxicity based upon the

use of non-specialized cell systems have been developed but have not yet been adequately validated.

(2) Further attention must be given to refining the choice of cell types, culture conditions and end-points as a first step toward standardization of techniques. In particular, the use of serum-free media should be encouraged.

(3) The Workgroup noted recent developments in organizing large-scale validation studies for selected methods. Efforts in this respect are to be encouraged with particular attention focused on the reproducibility and comparability of results, comparative testing using *in vivo* and *in vitro* techniques in parallel, and by testing large series of compounds for which comprehensive animal and human toxicological data already exist.

(4) There is a need to better determine the significance of specific end-points in all systems as they relate to *in vivo* toxicity.

REFERENCES

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