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Review

Proposal to improve vertebrate cell cultures to establish them as substitutes for the regulatory testing of chemicals and effluents using fish

Kristin Schirmer *

Department of Cell Toxicology (CellTox), UFZ-Centre for Environmental Research Leipzig-Halle in the Helmholtz Association, Permoserstr. 15, 04318 Leipzig, Germany

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Abstract

Cultures of vertebrate cells are widely applied in mechanistic studies in human toxicology as well as in toxicity identification in ecotoxicology. As *in vitro* models, they display many advantages over whole animal experimentation, pertaining to such characteristics as availability, reproducibility and costs. As well, they satisfy the societal desire to reduce the number of animals in toxicology. For these reasons vertebrate cell models also appear to be a desirable replacement for animals in regulatory tests. Several vertebrate cell models are now accepted for regulatory purposes in human health sciences, with the test for photocytotoxicity using the 3T3 mouse cell line being one example. However, an *in vitro* alternative to whole animal tests has not yet been established for regulatory risk assessment in ecotoxicology. This review sets out to outline why such a replacement has not yet been possible and explores avenues to improve vertebrate cell cultures so that a replacement of whole animal tests could more likely be achieved. Inasmuch as fish is the most widely used non-mammalian vertebrate in risk assessment and regulation, focus will be on the replacement, by *in vitro* vertebrate models, of fish.

Keywords: Cell lines; *In vitro* alternatives; Fish tests; Chemical regulation; Effluent testing

Contents

* Tel.: +49 341 235 2699; fax: +49 341 235 2434.
E-mail address: Kristin.Schirmer@ufz.de.

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1. Introduction

Vertebrate cell cultures are *in vitro* models. The term *in vitro* refers to keeping entities of an organism outside the living body in an artificial environment, in contrast to *in vivo*, i.e. in the organism. According to the use of *in vitro* terminology suggested by Schaeffer (1990), vertebrate cell lines arise from primary cultures. Primary cultures start from cells, tissues or organs taken directly from organisms. If a primary culture can be divided into new culture vessels and successfully propagated, it becomes a cell line. A cell line may be propagated a limited number of times, in which case it is finite, or indefinitely, in which case it becomes an immortal or continuous (or permanent) cell line. Mammalian cell lines are finite or continuous whereas most fish cell lines appear to be continuous (Bols et al., 2005).

The value of vertebrate cells in toxicological research began to be recognized in the late 1960s/1970s. At first, mammalian cell cultures were used to identify and understand potential effects posed to humans by chemicals in general (reviewed in Rees, 1980). In light of the central role of the fresh water environment in receiving, accumulating and distributing potentially hazardous substances, it was not surprising that the assessment of water quality soon emerged as another important area of application. Richardson et al. (1977) evaluated a mammalian cell culture assay for estimating the water quality of oil-refinery effluents. Several studies involving mammalian cells were performed by health laboratories and water boards (reviewed in Hunt et al., 1986). Rachlin and Perlmutter (1968) pioneered the use of a fish cell line as an indicator of toxicity by individual aquatic contaminants to fish. Finally, Ahne (1985) was the first to propose the use of fish cell lines as alternative to the fish lethality test in the monitoring of industrial effluents.

The philosophy underlying the application of vertebrate cells for predicting the toxicity in whole animals is that any interaction of a substance with an organism is initiated at the level of the cells. From cells, alterations can translate to changes in tissue or organ function and finally impact on whole organisms (Fig. 1). Based on the central role of cells in the expression of toxicity, several mammalian *in vitro* models have received regulatory acceptance by the Organisation for Economic Co-operation and Development (OECD) as alternatives to whole animal tests in human health science. The mouse cell line-based 3T3 Neutral Red Uptake Phototoxicity Test, for example, has been validated for the determination of toxicity elicited by chemicals in the presence of ultraviolet radiation (Spielmann et al., 2000).

Beside their potential to replace or reduce animals in toxicity tests, vertebrate cell cultures have several advantages compared to whole animal tests. Large numbers of potentially toxic substances can be screened rather quickly in multi-well plates, which can be analysed rapidly with, e.g. fluorescent multi-well plate readers. Little test substance is needed and thus less toxic waste produced. As well, cells can help to identify the mechanisms underlying a toxic response. If, for a particular purpose, a suitable continuous cell line can be used, a donor animal is never again needed. Based on these advantages, the role of vertebrate cell cultures is expected to significantly increase, beyond human health sciences, in environmental regulations. Specifically, the European Commission encourages the development and application of alternatives to animal tests in order to allow the new European legislation on the Registration, Evaluation and Authorisation of Chemicals (REACH) to be executed in an ethically and financially acceptable manner (Castaño et al., 2003).

Fish are the dominant vertebrate species for the regulatory evaluation of ecotoxicity and are afforded the

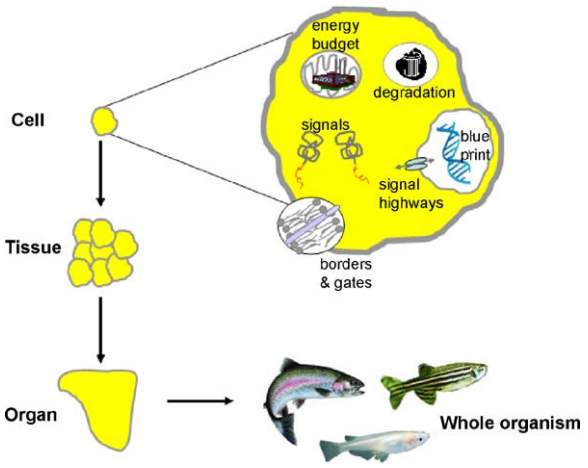

Fig. 1. Examples of sensitive target sites within vertebrate cells from which alterations can translate into impaired functions in whole organisms.