Available online at www.sciencedirect.com
SCIENCE DIRECT®
Regulatory Toxicology and Pharmacology 43 (2005) 219–224
Regulatory Toxicology and Pharmacology
www.elsevier.com/locate/yrtph

Ensuring quality of in vitro alternative test methods: Issues and answers

Kailash Gupta [a], Amy Rispin [b,*], Katherine Stitzel [c], Sandra Coecke [d], John Harbell [e]

[a] US Consumer Product Safety Commission, Bethesda, MD, USA
[b] US Environmental Protection Agency, Office of Pesticide Programs (7506C), Washington, DC, USA
[c] Consultant, West Chester, OH, USA
[d] European Center for the Validation of Alternative Methods, Institute for Health and Consumer Protection, European Commission Joint Research Center, Ispra, Italy
[e] Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA

Received 23 August 2004
Available online 15 August 2005

Abstract

Many in vitro and ex vivo methods have been developed or are under development to reduce or replace animal usage in toxicity tests. Consistent with the goal of obtaining scientifically sound test data for hazard and risk assessment of chemicals, changes are being made in current policies and procedures to facilitate the acceptance of data developed using these methods. National and international organizations are developing policies and standards for scientific practice to assure quality in implementation of in vitro methods. Consensus is developing in the scientific community for the quality control measures needed for in vitro methods; including appropriate controls, data reporting elements, and benchmarks to be identified in test guidelines so that the potential risks of chemicals can be reviewed and reliably assessed. Additional guidance to the OECD's Good Laboratory Practice principles [Organization for Economic Cooperation and Development (OECD), 2004. Advisory Document of the Working Group on Good Laboratory Practice: The Application of the Principles of GLP to in vitro Studies. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 14 (ENV/JM/MONO(2004)26). Paris, France] will help to ensure that in vitro tests used for regulatory purposes are reproducible, credible, and acceptable. Generic test guidelines incorporating performance standards are being written to allow acceptance of proprietary test methods by regulatory agencies and to provide assurance that any in vitro system performs over time in a manner that is consistent with the test system as it was originally validated.
Published by Elsevier Inc.

Keywords: Toxicology; In vitro; Alternative; Methods; Quality; Assurance; Control; Testing kits

1. Introduction: in vitro alternatives to animal testing including proprietary test methods

Legislative mandates for chemical control in the United States and other countries require submission of credible scientific data for use in assessing the hazards and potential risks of chemicals to humans, wildlife, and the environment. The requirement that evaluations of chemicals be based on safety test data of sufficient quality, rigor, and reproducibility is a basic principle in such legislation. Historically, in vivo tests in laboratory animals have formed the foundation of hazard and risk assessment. Good science, applied in these regulatory programs, calls for incorporation of the latest scientific advances, including non-animal methods such as cell and tissue culture systems, and high-throughput methods such as toxicogenomics and proteomics. Such methods may also serve as testing alternatives to current

* Corresponding author.
E-mail address: amy.rispin@epa.gov (A. Rispin).

0273-2300/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.yrtph.2005.03.010

methods that refine, reduce, or replace animal use while providing a comparable or better level of protection of human health or the environment.

Many in vitro or ex vivo and other non-animal methods have been developed or are currently under development to replace animal tests (ECVAM, 2002) or to allow direct assessment of chemical effects in human cells or tissue components. In vitro test systems pose different issues, as discussed below, regarding their quality and performance than commonly used animal methods (Rispin et al., 2004).

When any new test method is developed, test parameters are standardized so that laboratories can obtain consistent results. Validation involves systematic laboratory studies performed on a set of common reference chemicals to determine the new test's reliability in terms of intra- and inter-laboratory variability, and to assess how well it functions for various chemical classes. In the United States, the Interagency Coordinating Committee for Validation of Alternative Methods (ICCVAM) provides for review and assessment of the validity of the new toxicology tests including non-animal alternative test systems and proprietary test methods (ICCVAM, 1997). Thereafter, each regulatory agency determines if data generated using the new method are acceptable for its mandate.

The European Center for Validation of Alternative Methods (ECVAM) has been established to facilitate development of non-animal tests for the European Union. ECVAM also assesses the reliability and relevance of such tests for European regulatory mandates (ECVAM, 1995). Both ICCVAM and ECVAM are directed to seek alternative tests which reduce, refine, or replace animal testing.

Once a new in vitro method is validated and accepted for regulatory use, companies and regulatory authorities making decisions from the data need assurance that it will continue to perform in a manner consistent with the test system as it was originally validated. Stability of performance of the in vitro system is needed: over time; with any change in components of the test system; with any change in test system manufacturers; and with variations in interpretation of in vitro methods described by generic guidelines. In addition, testing laboratories must use good scientific practices, as well as appropriate calibration and standardization methodology established by the various technical disciplines appropriate to the elements of their assay system. For example, in vitro assays using cells in culture should incorporate use of good microbiological practices.

Some in vitro assays include a bioconstruct or ex vivo component that acts as the target tissue for the toxicological effect of concern. Such assays are based on a prediction model that relates the test endpoints to the toxic effect of concern. The bioconstruct can be cellular, non-cellular, or tissue construct. Tissue constructs, often using materials derived from humans, are designed to model the toxicology of cells or tissues and replicate the in vivo responses to chemical exposure. Test developers determine the performance of the assay for an array of chemical substances and exposure conditions and submit this information to the validating organization. All of the elements of the assay work as a single package for purposes of validation of the assay for its use to fulfill regulatory testing requirements. Once the assay system is validated, quality control, to determine the quality of the bioconstruct and associated reagents, is an essential element for any regulated study so that results of the assay can be reliably used in hazard and risk assessment and can be compared with data from previous studies within a laboratory and from one laboratory to another. Because these systems can be exquisitely sensitive to small changes in method or components, such systems must be well defined and function reproducibly.

Additional quality issues arise when the in vitro methods are developed, validated, and registered by manufacturers for commercial marketing as proprietary test methods. OECD[1] and United States agencies are writing generic descriptions of proprietary test methods; this allows other companies to enter the marketplace. Acceptance of these generic methods is based on validation data obtained using the proprietary version. European Union test guidelines use a similar procedure, following ECVAM review of the validation of in vitro methods. Both regulatory agencies and the users of these test systems need a process to ensure that "me-too" test kits developed according to the generic descriptions produce results similar to those obtained using the system originally validated and accepted.

2. Current methods for ensuring quality using Good Manufacturing Practice and Good Laboratory Practice and their applicable for new in vitro methods

In the United States, the Food and Drug Administration's (FDA) Good Manufacturing Practice (GMP) regulations contain provisions which describe how to ensure performance and consistency of in vitro methods when they are approved for commercial use by FDA (USFDA, 2003a,b). The GMP provisions from FDA contain criteria for setting performance standards for each assay but apply only if a proprietary test method falls under FDA authority as a device or kit for medical purposes. However, GMPs would not apply to in vitro and/or proprietary test methods that are marketed solely for use in fulfilling non-medical regulatory testing

[1] *Abbreviations used:* GCCP, Good Cell Culture Practice; GLP, Good Laboratory Practice; GMP, Good Manufacturing Practice; OECD, Organization for Economic Cooperation and Development.