Quality Considerations: Redefining Test Systems from Animals to Tissues and Beyond

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ABSTRACT

The use of non-whole animal test methods transforms the way regulatory requirements are applied in preclinical testing. Recent global regulatory initiatives emphasize the importance of transitioning to human relevant assays and test systems that do not use animals. When these methods are moved from research into the regulated arena, GLP principles must be followed. The GLPs were originally written in the 1970s, when the vast majority of regulated research was performed using animals as the test system. Current innovative, alternative test systems include *ex vivo* tissues, manufactured biological systems, three dimensional tissue constructs, and cell cultures maintained in dynamic flow bioreactors. Each type of alternative test system raises new quality and compliance points to consider when used within a regulatory context. Just as the applications of these methods have advanced with regulatory acceptance, the quality control and compliance of these test systems must also progress.

INTRODUCTION

The Organization for Economic Cooperation and Development (OECD) define a test system as "…any biological, chemical or physical system or a combination thereof used in a study," in the Principles of Good Laboratory Practices as revised in 1997 (OECD, 1997). The US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) both define a test system as "…any animal, plant, microorganism, or subparts thereof to which the test or control article is administered or added for a study," (FDA 21 CFR 58) and (EPA 40 CFR 160). The US EPA and FDA further include "…appropriate groups or components of the system not treated with the test or control articles," as part of the test system. Good Laboratory Practices were established at a time when regulated safety and efficacy studies were almost exclusively performed on animal test systems. The US FDA and EPA, as well as the OECD GLPs reflected this status quo. In the early 1990's the US EPA revised their GLPs to reflect the broader scope of work covered by their regulations. While the FDA was largely focused on health testing, where animals were the majority of the test systems used, the scope of the EPA FIFRA GLPs included the use of plants and soil samples as test systems and the regulations were revised accordingly. Since that time, many assays that do not include dosing whole animals have completed validation evaluation and have received OECD Test Guidelines. The table below provides a list of validated toxicological test methods that have received OECD Test Guidelines where animals, plants, and soil samples are not used as the test system.

Ex Vivo

OECD TG 437	Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying Ocular Corrosives and Severe Irritants	Bovine Cornea
OECD TG 438	Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants (ICE)	Whole Globe Chicken Eye
OECD TG 428	Skin Absorption: *In Vitro* Method	Non-viable or viable human and animal skin

Cell Culture

OECD TG 460	Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants	MDCK CB997 cell culture
OECD TG 491	Short Time Exposure *In Vitro* Test Method for Identifying i)Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	Statens Seruminstitut Rabbit Cornea (SIRC) cell culture
OECD TG 432	*In Vitro* 3T3 Neutral Red Uptake Phototoxicity Test	Balb/C 3T3 Cells
OECD TG 442D	*In Vitro* Skin Sensitization: ARE-Nrf2 Luciferase Test Method (KeratinoSens™)	KeratinoSens™ cell line
OECD TG 442E	*In Vitro* Skin Sensitization: Human Cell Line Activation Test (h-CLAT)	THP-1 cells

Manufactured Human Tissue Models

OECD TG 492	Reconstructed Human Cornea-like Epithelium (RhCE) Test Method for Identifying Chemicals not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage	3-dimensional RhCE Tissue Constructs
OECD TG 431	*In Vitro* Skin Corrosion: Human Skin Model Test	3-dimensional RhCE Tissue Constructs
OECD TG 439	*In Vitro* Skin Irritation: Reconstructed Human EpiDermis Test Method	3-dimensional Reconstructed Human Epithelium (RhE)

In Chemico

OECD TG 435	*In Vitro* Membrane Barrier Test Method for Skin Corrosion	Biobarrier Matrix
OECD TG 442C	*In Chemico* Skin Sensitization: Direct Peptide Reactivity Assay (DPRA)	Cysteine or lysine peptide incubated with the test chemical

POINTS TO CONSIDER FOR ALL NON-ANIMAL TEST SYSTEMS

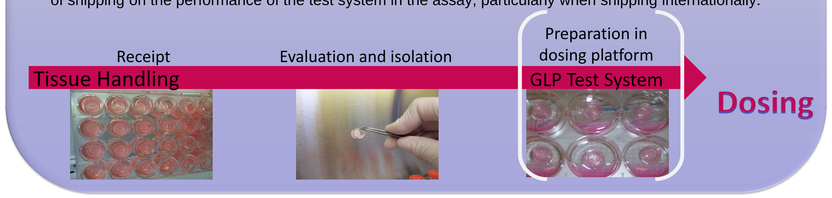
- **Justification for use**: Many non-animal testing options are available for use in place of what was, historically, a single animal test. Knowledgeable researchers should explain the rationale for selection of the test method based on the expected toxicity of their test material, the desired endpoint, and the regulatory authority to which the data will be submitted.
- **Consistent performance:** Concurrent positive and negative assay controls can be used to verify proper test system performance in each assay. Monitoring test system performance during each use is not an ethical concern for non-animal test methods and provides data on the consistency of the test system and reliability of the derived data over time.
- **Point of becoming the GLP test system:** Whereas in the typical animal testing paradigm, animals are received nearly ready for testing (save the quarantine and veterinary oversight, identification and randomization), many, if not all, non-whole animal test methods require various steps to transform cells, raw materials, reagents and test platforms/plastic ware into the defined GLP Test System used in subsequent test protocols. Thus it is critical that we clearly identify the criteria for when these raw cells and reagents can be declared the Test System. Quality processes should be used in these manipulations and compliant documentation should be kept. GLP test system requirements are met once the *in vitro* system has been prepared and is presented in the final platform used for dose exposure.

CELL CULTURE

- **Preparation of Test System**
  - Source
    - Purchase cells from a reliable source and assure there is documentation on the identification of the cell line to protect against inadvertent use of incorrect cell types or strains, e.g. HeLa.
    - Origin of the cell line should be documented (e.g., primary cells or tissues with defined donor characteristics) along with the methods for obtaining the cells (derived from tissue explants, biopsies of normal or cancer tissues, gene transfer by plasmid transfection or virus transduction)
  - Sterility: Cell lines used should also be shown to be free of mycoplasma contamination at the time of purchase and throughout maintenance and use.
  - If applicable, state of differentiation should be documented prior to use in the assay.
  - This documentation carries forward and satisfies the GLP requirement for identification of the test system when referenced in the study report.
- **Cell Line Handling Documentation:** Although not part of a specific study, records should be kept documenting the handling and treatment of the cell culture prior to preparation in the final platform for use in the assay
  - chronology of custody
  - passage number
  - culture conditions and sub cultivation intervals
  - freezing/thawing conditions
  - Track personnel, media lots and expiry, confluency on all splits and other culture manipulations in house prior to use in the assay

MANUFACTURED HUMAN TISSUE MODELS

- **Preparation of the Test System:** The vendor should retain documentation on origin of the initial cells used for processing and creation of the tissue construct. A functional characterization of the tissue should be performed to assure it satisfies the performance standards necessary for the test method.
- **Vendor Audit:** A scientific and quality systems audit of the vendor should be performed to assure the methods of the manufacturing process are well documented, batch specific production records are retained, personnel are adequately trained, and all other elements of a high quality manufacturing process are present.
- **Quality Control:** The vendor should have quality criteria that must be met for each batch of tissue prior to release for use. The release criteria should be sufficient to assure proper structure and function for use in the test method.
- **Shipping Validation:** Potential effects of shipping conditions on the biological system should be known and controlled as much as possible. A formal shipping "validation" or test may be considered to examine the effect of shipping on the performance of the test system in the assay, particularly when shipping internationally.

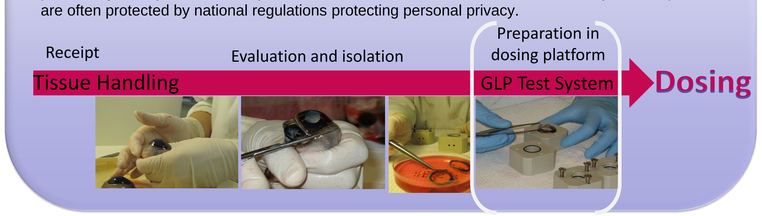


REFERENCES

- The Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice ENV/MC/CHEM(98)17
- U.S. Food and Drug Administration Good Laboratory Practice for Non-clinical Studies, 21 CFR- Part 58
- U.S. Environmental Protection Agency Good Laboratory Practices 40 CFR – Part 160, Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)
- U.S. Environmental Protection Agency Good Laboratory Practice Standards; Final Rule 40 CFR Part 792, Toxic Substances Control Act (TSCA)

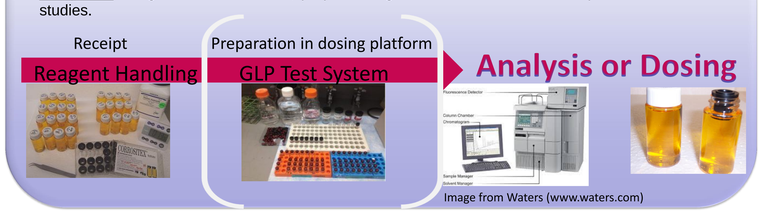
EX VIVO

- **Preparation of Test System:** Particularly with *ex vivo* test systems, a great deal of processing and preparation is performed prior to identifying the test system and confirming its' suitability for use in an assay. The entirety of the *ex vivo* tissue received is often not what is dosed in a test method, i.e. not the test system. Care should be taken to track the preparation of the test system, but the GLP definition of the test system is not met prior to 1) meeting predefined acceptance criteria and 2) integration with the dosing platform.
- **Identification of test system:** OECD GLPs 5.2.3. require "records of source, date of arrival, and arrival condition of test systems". *Ex vivo* systems derived from tissue byproduct from abattoirs will likely not have additional information available that is typical for whole animal test systems, such as age and gender. This information, although provided as a reference for usually acceptable *ex vivo* tissues in many OECD guidelines, is not as critical a measurement of test system suitability as protocol defined pre-dosing test system suitability measurements. For human based *ex vivo* test systems, specific data are often protected by national regulations protecting personal privacy.



IN CHEMICO

- **Preparation of the Test System:** Often the most difficult of *in chemico* studies is determining what actually meets the GLP definition of "test system". It is often a combination of the test material and a reagent that are evaluated over time or using specific analytical techniques.
- **Reagents:** Reagent characterization, proper storage and use have elevated importance in *in chemico* studies.



FUTURE IMPLICATIONS

- Inclusion of the "platform" or apparatus necessary to support proper test system functionality or dosing over the length of test material exposure will become more necessary to fully evaluate the quality of *in vitro* test systems as next generation systems like "organ" and "human on a chip" migrate into use within a regulated context.
- It may be prudent to modify the definition of test system to include the apparatus used for exposure so the quality of all components can be evaluated. The following language is suggested for inclusion in the GLP regulations to expand the current definition of test system: "*Test system* means any animal, plant, microorganism, or subparts thereof **presented in its finalized platform or assay conditions** to which the test or control article is administered or added for study. *Test system* also includes appropriate groups or components of the system not treated with the test or control articles."
- It is the responsibility of the user of the test system to determine the correct test method and use the test system correctly, audit the vendor, train technical staff, document test system preparation and each stage in the study's execution, establish and follow defined acceptance criteria when selecting suitable test systems, and employ concurrent controls and benchmarks.