The Final EUTOXRISK Symposium 33

The ToxTemp Document

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Background Information

Bench Marks

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Template for the Description of Cell-Based Toxicological Test Methods to Allow Evaluation and Regulatory Use of the Data

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The majority of toxicity tests still falls into the category of non-guideline methods. Data from these tests may be used to support regulatory decisions or to guide strategies for the assessments of compounds during research and development, if they fulfill basic requirements concerning their relevance, reproducibility and predictivity. Only a method description of sufficient clarity and detail allows interpretation and use of the data. Considering the fact that regulators were increasingly faced with data from non-guideline studies, the OECD provided the guidance document 211 (GD211) on method documentation for the purpose of safety assessment. The guidance leaves considerable uncertainty for scientists not familiar with regulation as to what level of detail would be required, and how individual questions are to be answered. Moreover, little attention was given to the description of the test system (i.e. cell culture) and the steps leading to its generation. To address these issues, an annotated toxicity test method template (ToxTemp) has been developed to (i) fulfill all requirements of GD211, (ii) to guide the user concerning the types of answers and information required, (iii) to include acceptance criteria for test elements, and (iv) to define the cells sufficiently and transparently. The fully annotated ToxTemp is provided here, together with reference to a database with exemplary descriptions of > 20 cell-based tests.

GD 211 chapter and chapter name		Information to be found in chapter(s) of ToxTemp
1.	**General information**	
	Assay Name (title) / Summary / Method description authors / Developer(s) laboratory and contact details / Date of assay development and/or publication / Reference(s) to scientific papers / Information about the assay in relation to proprietary elements / Information about the throughput / Status of method development and uses / Abbreviations and Definitions	1.1; 1.2; 2.2; 2.3; 2.5; 2.6; 2.7; 4.8; 5.10; 8.1; 9.1; 9.4; 9.5; 10.2; 11.4
2.	**Test Method Definition**	
2.1	Purpose of the test method	4.7; 8.1; 8.7; 9.2; 9.3
2.2	Scientific principle of the method	4.7; 5.5; 8.1; 9.2; 9.3
2.3	**Tissue, cells or extracts utilised in the assay and the species source**	**3.1 – 3.7; 4.2 – 4.8**
2.4	Metabolic competence of the test system	4.5
2.5	Description of the experimental system exposure regime	5.1; 5.6; 5.7; 5.10; 6.4; 6.7; 8.6; 11.3
2.6	Response and response measurement	1.2; 5.2; 5.3; 7.3; 7.4; 8.1; 9.3
	• *Response here makes reference to any biological effect, process or activity that can be measured*	*5.2 Endpoint(s) of the test method; 1.2 Abstract; 8.1 Scientific principle, test purpose and relevance; 9.3 Steps towards mechanistic validation*
	• *Specify precisely and describe the response and its measurement*	*5.2 Endpoint(s) of the test method; 5.3 Overview on analytical method(s) to assess test endpoint(s)*
	• *Specify the precise response or activity investigated as applicable e.g. "IC50"*	*7.3 Raw data processing to summary data*
	• *And how it is calculated*	*7.4 Curve fitting*
2.7	Quality / Acceptance criteria	3.2; 3.4; 4.2; 4.3; 5.4; 5.5; 5.6; 5.7; 6.7; 7.1; 7.5; 8.4; 9.5
2.8	Known technical limitations and strengths	5.8; 8.6; 8.7
2.9	Other related assays that characterise the same event as in 2.1	6.9
3.	**Data interpretation and prediction model**	8.2; 8.3; 9.2; 9.3
3.1	Assay response(s) captured in the prediction model	5.2; 8.2
3.2	Data analysis	7.3; 7.4; 8.4
3.3	Explicit prediction model	8.2
3.4	Software name and version for algorithm/prediction model generation	7.4
4.	**Test Method Performance**	
4.1	Robustness of the method	8.4; 10.2
4.2	Reference chemicals/chemical libraries, rationale for their selection and other available information	5.5; 5.6; 5.7; 8.3; 8.6
4.3	Performance measures/predictive capacity	7.4; 8.3; 9.4
4.4	Scope and limitations of the assay	8.2; 8.6; 8.7
5.	**Potential Regulatory applications**	8.1
5.1	Context of use	8.2; 8.4; 8.6; 8.7
6.	**Bibliography**	9.1
7.	**Supporting information**	2.8

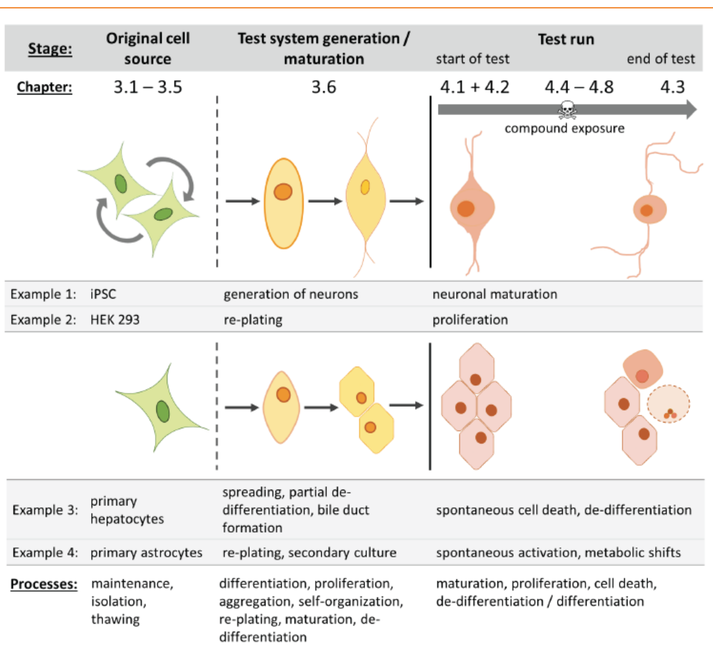


Figure 1: Documentation of cell culture stages relevant for their use in an* in vitro *test method
The ToxTemp presented here assesses test system features and the according acceptance criteria (AC) at different stages. In chapter 3, the original source of cells and their characteristics (green cells) as well as their differentiation/maturation (yellow cells) are covered. Chapter 4 focuses of the test system stages at the beginning of the test, i.e. at the start of chemical exposure, and at the final stage (end of test; red cells). Four examples of cellular test systems and their stages during test preparation and testing are given. In addition, exemplary processes (e.g., proliferation) which can change a test system are indicated and which should therefore be documented and taken into account for a comprehensive test method documentation.

The technology

- Template for a toxicological, cell based test method description (ToxTemp)
- 1. Overview
  - 1.1 → Descriptive full-text title
  - 1.2 → Abstract
- 2. General information
  - 2.1 → Name of test method
  - 2.2 → Version number and date of deposition
  - 2.3 → Summary of introduced changes in comparison to previous version(s)
  - 2.4 → Assigned data base name
  - 2.5 → Name and acronym of the test depositor
  - 2.6 → Name and email of contact person
  - 2.7 → Name of further persons involved
  - 2.8 → Reference to additional files of relevance
- 3. Description of general features of the test system source*
  - 3.1 → Supply of source cells
  - 3.2 → Overview of cell source component(s)
  - 3.3 → Characterization and definition of source cells
  - 3.4 → Acceptance criteria for source cell population
  - 3.5 → Variability and troubleshooting of source cells
  - 3.6 → Differentiation towards the final test system
  - 3.7 → Reference / link to maintenance culture protocol
- 4. Definition of the test system as used in the method
  - 4.1 → Principles of the culture protocol
  - 4.2 → Acceptance criteria for assessing the test system at its start
  - 4.3 → Acceptance criteria for the test system at the end of compound exposure
  - 4.4 → Variability of the test system and troubleshooting
  - 4.5 → Metabolic capacity of the test system
  - 4.6 → Omics characterization of the test system
  - 4.7 → Features of the test system that reflect the *in vivo* tissue
  - 4.8 → Commercial and intellectual property rights aspects of cells
  - 4.9 → Reference / link to the culture protocol
- 5. Test method exposure scheme and endpoints
  - 5.1 → Exposure scheme for toxicity testing
  - 5.2 → Endpoint(s) of the test method
  - 5.3 → Overview of analytical method(s) to assess test endpoint(s)
  - 5.4 → Technical details (of e.g. endpoint measurements)
  - 5.5 → Endpoint specific controls / mechanistic control compounds (MCC)
  - 5.6 → Positive controls
  - 5.7 → Negative and unspecific controls
  - 5.8 → Features relevant for cytotoxicity testing*
  - 5.9 → Acceptance criteria for the test method
  - 5.10 → Throughput estimate*
- 6. Handling details of the test method
  - 6.1 → Preparation / addition of test compounds
  - 6.2 → Day-to-day documentation of test execution
  - 6.3 → Practical phase of test compound exposure
  - 6.4 → Concentration settings
  - 6.5 → Uncertainties and troubleshooting*
  - 6.6 → Detailed protocol (SOP)
  - 6.7 → Special instrumentation
  - 6.8 → Possible variations*
  - 6.9 → Cross-reference to related test methods*
- 7. Data management
  - 7.1 → Raw data format
  - 7.2 → Outliers
  - 7.3 → Raw data processing to summary data
  - 7.4 → Curve fitting
  - 7.5 → Internal data storage*
  - 7.6 → Metadata
  - 7.7 → Metadata file format
- 8. Prediction model and toxicological application
  - 8.1 → Scientific principle, test purpose and relevance
  - 8.2 → Prediction model
  - 8.3 → Prediction model setup
  - 8.4 → Test performance
  - 8.5 → *In vitro – in vivo* extrapolation (IVIVE)
  - 8.6 → Applicability of test method*
  - 8.7 → Incorporation in test battery*
- 9. Publication / validation status
  - 9.1 → Availability of key publications
  - 9.2 → (Potential) linkage to AOPs*
  - 9.3 → Steps towards mechanistic validation*
  - 9.4 → Pre-validation or validation*
  - 9.5 → Linkage to (e.g. OECD) guidelines / regulatory use*
- 10. → Test method transferability*
  - 10.1 → Operator training*
  - 10.2 → Transfer*
- 11. → Safety, ethics and specific requirements
  - 11.1 → Specific hazards, issues of waste disposal
  - 11.2 → Safety data sheet (SDS)
  - 11.3 → Specific facilities / licenses
  - 11.4 → Commercial aspects / intellectual property of material / procedures*

- 1. Overview

1.1 Descriptive full-text title

Provide a descriptive title using normal language without technical terms or acronyms.

Example: "Assay to test compound-derived impairment in neurite outgrowth in human mature dopaminergic neurons (NeuriTox; UKN4)."

1.2 Abstract

Please describe in not more than 200 words the following:

Which toxicological target (organ, tissue, physiological/biochemical function, etc.) is modelled? (8.1)

Which test system and readout(s) are used? (4.1 and 5.2)

Which biological process(es) (e.g. neurite outgrowth, differentiation) and / or toxicological events (e.g. oxidative stress, cell death) are modelled / reflected by your test method? (8.1)

To which (human) adverse outcome(s) is your test method related or could be related? (8.1; 9.2 and 9.3)

Which hazard(s) do(es) your test method (potentially) predict? (8.1 and 8.6)

Does the test method capture an endpoint of current regulatory studies? (9.5)

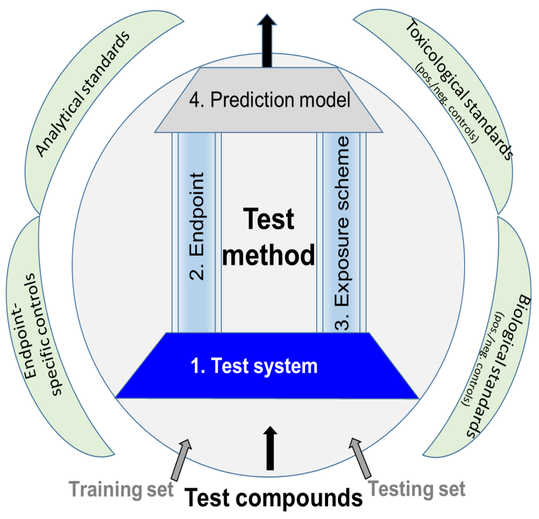
If the method has undergone some form of validation / evaluation, give its status. (9.4)

Note: this section should give an overview. Details can be found in the respective chapters, as indicated by numbers in brackets.

***Important note:** Look at the Appendix, before filling in the test method description form. Note: a test consists of several elements. If one is changed (e.g. the endpoint, duration of compound exposure, etc.), it requires a new submission with a different database name.*

For the description here, choose one strictly defined protocol / application, and refer all information to this setup. Possible variations can be indicated in chapter 6.8. If a variation (e.g. altered incubation time or other endpoint) is implemented, then such a variant needs to be deposited as new test.

If a section / question is not applicable for your test method, please indicate "not applicable". If there is no information, please indicate "no information available".



Application examples

- 8.3 Prediction model setup

How was the prediction model set up (using which testing set of chemicals for training of the model; using probing what kind of classifiers / statistical approaches)?

Has the prediction model been tested (what was the testing set of chemicals)? List chemicals or give n, if n > 50.

Is the process documented (publication)?

Does the prediction model (PM) apply to changes to both sides of controls (up / down)? If the PM is one sided (e.g. toxicants leading to a decrease vs. control), how are data in the opposite direction handled and interpreted? If the PM is two sided, do different rules, characteristics and interpretations apply to the two sides (e.g. is a decrease in viability, or an increase in viability both interpreted as effect / toxicity; are thresholds and performance characteristics to both sides the same?).

Note: for final values on accuracy, sensitivity and specificity, refer 8.4.

- 8.4 Test performance

Indicate here basic performance parameters or if possible preliminary estimates (label as such): Baseline variation (noise) within assays AND between assays.

What is the signal / noise ratio (signal / = standard positive control)?

Is the z-factor determined?

Give the specificity of the test method. How is it determined?

Give the sensitivity of the test method. How is it determined?

Give measures of the uncertainty of your test method. How are they determined?

What is the detection limit (required change of endpoint to become measurable)?

If available, give limit of detection (LOD) and limit of quantification (LOQ).

What are inter-operator variations?

Are there data of 'historical controls' over longer time period?

Note: assay parameters can only apply to one assay version. A standard version must be defined and referred to in all answers.

- 8.5 *In vitro – in vivo* extrapolation (IVIVE)

Describe parameters important for determination of free compound concentrations in the medium:

Give the lipid and protein content of the medium and the cells.

Give the volume of the cells.

Give volume (medium volume) and surface area of culture dish.

Is there information / literature on IVIVE strategies / data in the test?

Has the test been used earlier for IVIVE?

Are there special considerations relevant for IVIVE (e.g. potential for compound accumulation due to frequent medium changes and compound re-addition, glycoprotein (MDR1) expression, capacity for xenobiotic metabolism of test system)?

- 8.6 Applicability of test method*

Note: this refers to the biological and chemical applicability.

Which compounds is the test likely to pick up correctly, where is it likely to fail?

How does the test method react to mixtures and UVCBs?

Are there areas (according to industry sector, compound chemistry, physical-chemical properties) that need to be excluded from testing, or that are particularly suitable?

Which compound class cannot be detected (e.g. neurotransmitters for which the receptors are not expressed, endocrine disruptors in absence of respective pathway)?

Are there compounds interfering with the test system (e.g. fluorescing or coloured chemicals)?

- 7.1 Raw data format

What is the data format?

Raw data: give general explanation. Upload an exemplary file of raw data (e.g. Excel file as exported out of plate reader).

Give an example of processed data at a level suitable for general display and comparison of conditions and across experiments and methods.

Note: it is recommended that data formats suitable for most / all methods are pre-defined in collaborative projects, such as EU-ToxRisk or ToxCast.

If the file format is not proprietary or binary, please upload a template. This will help other users to provide their data in a similar way to the general data infrastructure.

Example as used in EU-ToxRisk: Excel sheet with columns specifying line number, assay name, date of experiment, identifier for reference to partner lab book, compound, concentration (in: -log[M]), line number of according control, number of replicates, endpoints, data of endpoint(s), etc.

- 7.2 Outliers

How are outliers defined and handled?

How are they documented?

Give the general frequency of outliers.

Note: if only processed data are reported, outlier information gets lost.

- 7.3 Raw data processing to summary data

How are raw data processed to obtain summary data (e.g. EC_{50}, BMC15, ratios, PoD, etc.) in your lab?

Describe all processing steps from background correction (e.g. measurement of medium control) to normalization steps (e.g. if you relate treated samples to untreated controls).

- 7.4 Curve fitting

How are data normally handled to obtain the overall test result (e.g. concentration response fitting using model X, determination of EC_{50} by method Y, use of EC_{50} as final data)?

How do you model your concentration response curve (e.g. LL 4 parameter fit) and which software do you use (e.g. GraphPad Prism, R, etc.)?

Do you usually calculate an uncertainty measure of your summary data (e.g. a 95% confidence interval for the BMC or a BMCL), and with which software?

Can you give uncertainty for non-cytotoxicity or no-effect?

How do you handle non-monotonic curve shapes, or other curve features hard to describe with the usual mathematical fit model?

- 7.5 Internal data storage*

How and how long are raw and other related data stored?

What backup procedures are used (how frequently)?

How are data versions identified?

- 7.6 Metadata

Note: metadata are for example: laser power, microscope objective, binning of camera, slit / filter of optical units, temperature cycle of PCR, all data that refer to instrument settings during data recording, suppliers of chemicals, software versions for data processing, types of dishes, media and consumables used, etc.

How are metadata documented and stored (lab book, excel files, left in machine, etc.)?

How are they linked to raw data?

What metadata are stored / should be stored?

- 7.7 Metadata file format

Give example of the metadata file (if available).

Note: also consider in this template to include fields which are variable, but cannot be completely specified in the protocol and could be changed without changing the readout (e.g. suppliers of chemicals).

HORIZON 2020

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www.eu-toxrisk.eu/