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GUIDANCE DOCUMENT FOR DESCRIBING NON-GUIDELINE IN VITRO TEST METHODS

Series on Testing and Assessment
No. 211

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OECD Environment, Health and Safety Publications

Series on Testing and Assessment

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FOREWORD

The guidance document for describing non-guideline *in vitro* methods was initiated in 2013 by the Advisory Group on Molecular Screening and Toxicogenomics (EAGMST), operating under the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) at the OECD. The need for such guidance emerged with the increasing number of non-standard *in vitro* methods often using elaborated technologies to produce a large amount of data. A draft of the document was brought to the attention of the EAGMST and WNT in October 2013 for review. In December 2013, the draft was also discussed and commented on by the Validation Management Group for Non-Animal Testing. A revised draft was available to the WNT meeting in April 2014 and circulated for a formal commenting round in July 2014. A revised draft was circulated for approval via written procedure to WNT in September 2014.

It is acknowledged that this is an area in rapid development, and there is limited experience at the moment on best practice to describe this type of new *in vitro* methods. This first edition of the guidance document may thus need to be revised in the near future.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

GUIDANCE DOCUMENT FOR DESCRIBING NON-GUIDELINE *IN VITRO* TEST METHODS

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GUIDANCE FOR DESCRIBING NON-GUIDELINE *IN VITRO* TEST METHODS TO FACILITATE THEIR CONSIDERATION IN REGULATORY APPLICATIONS

INTRODUCTION

Purpose

1. Regulators are increasingly faced with data from non-guideline studies, especially from novel *in vitro* assays, in particular for priority setting of substances that require evaluation and may require testing but also within the context of their safety assessments. The rapid timelines for development of such new assays and the lack of transferability of certain technologies do not make these assays easily amenable to Test Guideline development and use. Nevertheless, the information generated from these methods can be useful at several levels in the safety assessment process. This guidance outlines the information that ideally should be provided by developers to describe non-guideline in vitro methods in order to facilitate an assessment of the quality of data produced and the potential utility in regulatory applications. It has been developed in the context of the work of the OECD Advisory Group on Molecular Screening and Toxicogenomics, and its programme on the development of Adverse Outcome Pathways (AOPs).

2. The purpose of this guidance is to harmonise the way non-guideline in vitro methods are described and thereby facilitate an assessment of the relevance of test methods for biological activities and responses of interest, and an assessment of the quality of data produced, irrespective of whether these tests are based on manual protocols or assay protocols adapted for use on automated platforms or high-throughput screening systems (HTS). This guidance outlines the elements considered relevant for providing a comprehensive description of an in vitro method to facilitate the interpretation of results and support scientifically defensible fit-for-purpose applications, so as to be suitable for their use in decision making processes by regulators in particular or by the scientific community and end users in general.

3. This guidance is neither intended to be prescriptive nor does it endorse a particular structure for reporting the information. On the contrary, the structure should be both flexible and transparent and the example given below merely illustrates the type of information that could be helpful to characterise a particular method. The completeness of the information provided will vary depending on the level of development of the method under consideration and this in turn will impact its use in different regulatory applications (see Section 5). Note the structure here outlines the information about a given method, not how the results generated from such methods should be structured, recorded and reported. Work is additionally underway to develop an OECD Harmonised Template for capturing results from in vitro assays, so named OHT 201. Finally, this document does not provide guidance on how the information generated with such methods can be used for regulatory purposes.

Background

4. In the past, the development of alternative methods and in particular *in vitro* alternative methods has been largely motivated by the possibility to model or predict **downstream health effects** observable in situ (so-called "apical endpoints"), be it by one method only (=stand-alone alternative) or by a group of assays used either in conjunction ("test battery") or in a strategic sequence ("testing strategy"), or to provide a mechanistic understanding of responses induced by chemicals, as a supplement animal experiments. Examples include methods to evaluate skin corrosion and irritation potential, which have resulted in specific OECD Test Guidelines. The validation process that such methods have followed is described in more detail in OECD Guidance Document 34 (guidance document on the validation and international acceptance of new or updated test methods for hazard assessment[1]). Scientific principles of assay validation described in Guidance Document 34 are relevant for many types of assays, irrespective of whether the predictions are for apical or for intermediate effects. However since the publication of the NRC Report[2] in 2007, initiatives such as Tox21[3] and the US EPA's ToxCast[4] have sought to exploit emerging technologies such as high throughput screening (HTS) and high content screening (HCS) assays to generate data. Such assays are sometimes termed "next generation" in vitro assays. There are currently a number of challenges in using HTS and HCS data in regulatory activities. A major challenge is the lack of development of standard guidance based on traditional validation, which might not be appropriate or relevant for such methods, not least since the assays might not be easily transferable to third laboratories due to very specific equipment (i.e. robotic platforms). In such cases assessment of transferability and between-laboratory as foreseen in regular validation (GD 34) would be less relevant. Furthermore, the evolution of some of these assays might be too rapid to justify a full and lengthy formal validation process in view of the standardization and acceptance as OECD Test Guidelines. Also, it may happen that an assay may only have utility for a particular /limited chemical class or sector (e.g. alcohols, solids, etc.) which makes the assay useful to characterize but does not warrant a full validation and Test Guidelines development process.

5. Nervertheless, data generated by these methods, as well as by many other in vitro methods that may be early in their development stage or rapidly developing, not measuring an established regulatory hazard endpoint, or of narrow applicability(e.g., limited in the range of physical-chemical properties of chemicals tested) for a given hazard/biological endpoint, can be of practical value, e.g. to facilitate a better understanding of the mechanisms of action of a substance and thus could be used to a greater extent in a regulatory context..

6. The availability of novel technologies and new in vitro methods at early stages of development, including these "next-generation" *in vitro* methods, raises questions - how can these methods be practically utilised whilst ensuring that the resulting data are scientifically robust and interpreted in the appropriate context? It is critical to have some type of framework in place to help evaluate the uncertainties and interpreting such in vitro methods to assure scientific confidence. Anchoring such methods in the appropriate biological context (e.g. Adverse Outcome Pathway (AOP)) supports the examination of scientific uncertainties and should in turn help to inform their use in integrated approaches to testing and assessment (IATA) as well as chemical grouping and prioritisation. An example of such types of

[1] OECD (2005). Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. ENV Series on Testing and Assessment, No. 34, OECD Publishing.

[2] National Research Council, Toxicity Testing in the 21st Century: A Vision and a Strategy (June 2007), available at: http://dels.nas.edu/Materials/Report-In-Brief/4640-Toxicity-Testing

[3] US Tox21 Program, more information from: http://epa.gov/ncct/Tox21/

[4] US ToxCast Program, more information from: http://www.epa.gov/comptox/toxcast/

regulatory applications are being described in more detail in another guidance that is currently undergoing development (ref to Guidance on IATA for skin sensitization, in preparation).

7. This first edition of the guidance document is based on experience from experts in OECD countries/regions, industry and animal welfare groups in applying good practice when describing high-content *in vitro* methods. As more experience is gained in future, especially with more high-throughput and high content assays, the guidance may be updated to augment its applicability to those new types of assays, as appropriate.

Scope and use

8. This guidance provides a framework for describing existing knowledge about a method, termed **Method Description**, and abbreviated to **MD**. The MD structure below outlines the type and level of information that would be ideally captured. It is based on principles outlined in the IOM Framework (2010) [see http://nap.edu/catalog.php?record_id=12869] and uses the same information elements for in vitro methods indicated in OECD Guidance 34 and in an existing Streamlined Summary Document (OECD Series on Testing and Assessment Nr. 180; Streamlined Summary Document Related to the Fluorescein Leakage (FL) Test Method for Identification of Ocular Corrosives and Severe Irritants (TG 460)). It also mirrors many of the same information elements that form the basis of the Joint Research Centre's (JRC) DataBase service on ALternative Methods (DB-ALM) [see http://ecvam-dbalm.jrc.ec.europa.eu/] as well as characteristics defined by the OECD Thyroid Scoping Group[5].

9. This guidance is not intended to duplicate or replace OECD Guidance Document 34 (GD34), rather it is complementary and addresses methods that have not been officially validated.

10. There is of course flexibility in the use of the MD guidance document, depending on the level of development of individual methods, but also on the number of methods available that form a consistent set. In the case of a set of test methods representing a suite of key events on an AOP for example, some sections of the MD should be filled-in for each method (e.g. sections 1 and 2), while other sections may be filled in for the entire set as a whole rather than for each method under consideration.

11. This guidance document mainly targets test method developers as main users. Ultimately any completed MD should be stored in a publicly available repository/library of methods. This is beyond the scope of this guidance, although existing repositories such as JRC's DB-ALM may be helpful. In the repositories of MDs in countries/regions, the status, accountability for information provided in MDs, level of verification applied should be clearly stated to avoid misunderstanding.

12. The MD addresses information related to the purpose and scope of the assay, a description of the method components including protocol and reference chemicals, the stage of development of the assay, the

[5] OECD (2014), New Scoping Document on *in vitro* and *ex vivo* Assays for the Identification of Modulators of Thyroid Hormone Signalling, Series on Testing and Assessment No. 207, OECD Publishing, Paris.

quality /acceptance criteria, data interpretation and prediction model(s), performance including sensitivity[6] and predictivity[7].

13. In the MD example below, some of the information elements may not be relevant for a given assay, or are not known, or simply cannot be provided. In such cases, annotating with "no information available" will still be helpful for the end user to understand when evaluating the overall assay. It is also worth noting that some elements may be redundant or may be more readily populated based on the way in which an electronic repository has been structured e.g. particular fields in Section 1 are of note.

14. Although this guidance document does not prescribe conditions for filling-in MDs, when non-standard methods appear useful and relevant, jurisdictions may require further information in an MD format before using the methods or the data generated in a regulatory context. The use of MD is in the remit of countries/regulatory authorities in countries/regions. Regulatory authorities should be consulted for further information on the implementation and use of the MD.

1. General information

1.1 Assay Name (title): provide a short and descriptive title for the assay.

1.2 Summary: provide a summary of the assay features. E.g. "the effects of a chemical irritant on the opacity and permeability of a freshly isolated bovine cornea can be used to measure eye irritancy."

1.3 Date of MD: report the date of the first version of the MD (day/month/year). Example: "5 August 2013".

1.4 MD author(s) and contact details: indicate the name(s) and the contact details of the author(s) of the MD (first version of the MD).

1.5 Date of MD update(s) and contacts:

- **Date:** indicate the date (day/month/year) of any update of the MD. The MD can be updated for a number of reasons such as additions of new information (e.g. addition of new validation studies in section 7) or corrections to the existing information. Summarise briefly which information has been updated.
- **Contact:** indicate the name and the contact details of the author(s) of the updates if different from that in 1.4.

1.6 Assay developer(s)/Laboratory and contact details: indicate the name of the assay developer(s)/author(s)/laboratory(ies), and the corresponding contact details.

1.7 Date of assay development and/or publication: report the year of initial release/publication of the assay described in the current MD, indicate existence of standard operating procedures if they were made available to the public.

[6] Sensitivity: the proportion of all positive/active substances that are correctly classified by the test. It is a measure of accuracy for a test method that produces categorical results, and is an important consideration in assessing the relevance of a test method.

[7] Specificity: the proportion of all negative/inactive substances that are correctly classified by the test. It is a measure of accuracy for a test method that produces categorical results and is an important consideration in assessing the relevance of a test method.

1.8 Reference(s) to main scientific papers: list all bibliographic references to original paper(s) explaining the assay development. Any other reference such as references to validation datasets or prediction model development can be reported in field 6.0 "Bibliography". References should be publicly available or attached.

1.9 Availability of information about the assay in relation to proprietary elements: indicate whether the assay is proprietary or non-proprietary (to what extent is the assay method transferable or contains proprietary elements) and specify (if possible) what kind of information about the assay cannot be disclosed or is not available (e.g., chemical reference sets (training or test sets), prediction model). Example: "The assay is non-proprietary but the training and test sets are not available"; "The model is proprietary and the prediction model and associated data sets are confidential"; "Company XYZ's in vitro model for sensitisation may be transferable but the prediction model is confidential".
Obviously ensuring scientific confidence in an assay is facilitated if all information is transparent and can be disclosed.

1.10 Information about the throughput[8] of the assay: indicate the throughput of the assay to provide an indication of likely resource intensity e.g. low (manual assay, one chemical tested at a time), low-moderate, moderate, moderate-high, high throughput (e.g. in 96 well-plate and higher), and qualify with e.g. approximate number of chemicals/concentrations per run. If appropriate indicate whether a manual assay could be run in a higher throughput mode.

1.11 Status of method development and uses: compile information for the following sections if appropriate. Considerations could include:
i) Development status: Indicate if the assay is still under development, and the estimated timeline for completion as far as possible
ii) Known uses: Summarise the current and/or past use of the assay by different laboratories
iii) Evaluation study: Summarise the main conclusions or refer to individual protocol if available
iv) Validation study: Indicate participation in a formal validation study/studies and summarise the conclusions and their outcomes or refer to the individual protocol if available
v) Regulatory use: Provide details of any potential regulatory application and of the toxicological hazard endpoint being addressed by the assay.

1.12 Abbreviation and Definitions
If any terms were abbreviated or need specific definitions, they should be provided in this section.

2. Test Method Definition

2.1 Purpose of the test method
The claimed purpose and rationale for intended use of the method (e.g. alternative to an existing method, screening, provision of novel information in regulatory decision-making, mechanistic information, adjunct test, replacement, etc.) should be explicitly described and documented. The response measured in the assay should be put in the context of the biology/physiology leading to the *in vivo* response or effect.If the biological activity or response refers to a key event or molecular initiating event (MIE), provide a short description indicating firstly what key event within an existing or developing AOP, or in relation to a

[8] throughput means the amount of information or data passing through a system from input to output (e.g. experimental system, computer program) over a period of time.

mechanism or mode of action, the assay is aiming to characterize (i.e. which level of biological organization the assay may be attributed (e.g. sub-cellular, cellular, tissue, organ or individual), and secondly where the assay might fit in the context of an existing regulatory hazard (i.e. adverse outcome). E.g. the GSH assay[9] measures reactivity which characterises covalent binding, which is the MIE within the AOP for skin sensitisation; the relative binding affinity as measured in an estrogen receptor (ER) binding assay characterises the molecular initiating event (MIE) in an AOP for ER-mediated reproductive impairment. For definitions of an AOP and guidance in developing an AOP, see the Guidance Document No. 184; Guidance Document on Developing And Assessing Adverse Outcome Pathways.
In the absence of any AOP, provide an indication of the plausible linkage between the mechanism(s) the assay is measuring and the resulting hazard endpoint. More specific information can be captured in section 2.2 (Scientific principle of the method), e.g. "This assay addresses peptide reactivity as the MIE within the AOP for skin sensitisation by covalent binding by measuring depletion of synthetic heptapeptides containing either cysteine or lysine following a 24 hour incubation period."

2.2 Scientific principle of the method: provide the scientific rationale, supported by bibliographic references to articles, for the development of the assay. A summary description of the scientific principle including the biological/physiological basis and relevance (e.g. modeling of a specific organ) and/or mechanistic basis (e.g. modeling a particular mechanism by biochemical parameters) should be described. If possible, indicate what the anchor point is within an AOP.

2.3 Tissue, cells or extracts utilised in the assay and the species source: indicate the experimental system for the activity or response being measured. e.g. "freshly isolated bovine cornea";" Porcine TOP"; "Bovine lactoperoxidase"; "hER from extracts of human breast cancer cells"; "mERa from mouse uterine membranes". Provide information on whether materials are readily available commercially or whether materials are developed in the laboratory (e.g. cell suspensions from tissue). Indicate source/manufacturer of biological material used. Indicated whether cryopreserved biological material can be used or only freshly prepared.

2.3 Metabolic competence of the test system: describe and discuss the extent to which the test system can be considered metabolically competent, either by itself, or with the addition of an enzymatic fraction, if appropriate. Provide reference if available.

2.5 Description of the experimental system exposure regime: provide a summary description of the essential information pertaining to the exposure regime (dosage and exposure time including observation frequency) of the test compounds to the experimental system including information on metabolic competence if appropriate; number of doses/concentrations tested or testing range, number of replicates, the use of control(s) and vehicle. Also, describe any specialized equipment needed to perform the assay and measure the response. Indicate whether there might be potential solubility issues with the test system, and solutions proposed to address the issue.

2.6 Response and Response Measurement: response here makes reference to any biological effect, process or activity that can be measured. Specify precisely and describe the response and its measurement, e.g. corneal opacity measured using an opacitometer; half maximal activity concentration (AC50) derived from a competitive binding assay in human estrogen receptor assay or from the up-regulation of the proinflammatory antiangiogenic chemokine CXCL10.

Specify the precise response or activity investigated as applicable e.g. "IC50"; "half maximal activity concentration - AC50", "in vitro irritation score (IVIS) = mean opacity value + (15*mean permeability

[9] Glutathione assay, more information from: http://www.nature.com/nprot/journal/v1/n6/full/nprot.2006.378.html

OD490 value) is used to evaluate the irritation potential of a test material", and how it is calculated (e.g. IC50 using modified Hill equation).

2.7 Quality / Acceptance criteria: as appropriate, provide information on the availability of acceptance criteria and quality assurance as it pertains to the:

- Experimental data (storage/archiving), indicate unit of measurement of the raw data , not only transformed data
- Experimental system(s) used
- Equipment used, calibration program
- Availability of internal standards (e.g. positive and negative controls, reference chemicals, performance benchmarks
- Standards followed such as good cell culture practice, if relevant
- Criteria to accept or reject experimental data
- Limit of detection and limit of quantification, detection range.

2.8. Known technical limitations and strengths: specify any known technical limitations or strengths in running the assay. E.g. "Obtaining thyroid glands as a source of TPO may be an obstacle to the use of this assay in other laboratories" (see section 4.4. for the applicability of the assay). The assay may not be technically applicable to certain types or class of chemicals.

2.9 Other related assays that characterise the same event as in 2.1: Identify any related assays if known that may characterise the same key event as described in 2.1 e.g. GSH assay that measures reactivity and provide an indication of whether a MD has been prepared if possible. Make references to it, as appropriate.

3. Data interpretation and prediction model

If the data generated by the assay has itself or is already being used in a prediction model, provide here a brief summary and references for the prediction model. The prediction model, if available, may be derived from the assay itself or from a battery of assays i.e. how are the results of the assays/battery being summarised or interpreted to derive an outcome that can be used for a particular decision. E.g. the depletion results from lysine and cysteine peptide reactivity assays might be encoded in a prediction model to provide an overall indication of reactivity. The prediction model could be based on more than one assay outcome, if so, a MD for the associated assay(s) should be documented separately. It may be helpful to consider the intended purpose of the prediction model e.g. a desire to predict Y from assays that measure X starting from the step in the AOP that is being measured by the assays (X) and going to the "predicted outcome" step Y in the AOP. Please specify if this refers to key events as defined in AOPs.

3.1 Assay response(s) captured in the prediction model: Identify the response(s) from the given assay(s) that form(s) the basis of the prediction model. Note the response could be the same as specified in 2.2; if so, state "see 2.2".

3.2 Data analysis: Comment on the response value in terms of a boundary or range to provide a context for interpretation. E.g. putting into context what a negative value or >100% value might represent in a binding inhibition assay.

3.3 Explicit prediction model: Describe the prediction model. If the prediction model is too long or complex and cannot be practically reported here, include in this field a reference to a paper or a document where the prediction model is already described. This material can also be attached as supporting information in field 7.0.

3.4 Software name and version for algorithm/prediction model generation: Specify the name and the version of software applications (and date if relevant) used to derive the prediction model or to undertake the statistical processing.

4. Test Method Performance

4.1 Robustness of the method

This section should provide information on the robustness of the method i.e. the reliability of the experimental results and the prediction capability of the model used. Information on:

- within-laboratory repeatability and reproducibility,
- between laboratory transferability and reproducibility,

should be reported if known, or any other information relevant for the evaluation of the method depending on the method's purpose, including but not limited to, any analytical verification performed.

4.2 Reference chemicals/chemical libraries, rationale for their selection and other available information: Indicate whether the results for the reference chemicals (i.e. the "training set" chemicals used in the development and evaluation of the assay and its associated prediction model) are free and publically available in some form (e.g., published in a paper or a regulatory document, stored in a database) or appended to the current MD as supporting information (see field 7.0). If it is not available, explain why. e.g. "They are available and attached";"They are available but not attached (indicate reason)"; "They are not available because the data set is proprietary".

If the training set is being appended as supporting information (see field 7.0), indicate what information exists for the reference chemicals including the rationale for their selection if available; e.g. for mono-constituent substances: a) response values; b) Chemical names (common names and/or IUPAC names); b) CAS numbers or other identification number; c) SMILES and InCHI code if relevant; d) MOL files; e) Structural formula; f) Availability from commercial sources. In case of multi-constituent substances, UVCBs or mixtures, report the composition as far as possible. In case of specific types of chemicals (e.g. nanomaterials) for which additional characteristics may be relevant, report these characteristics/properties.

4.3 Performance measures/predictive capacity (if known): Report any goodness-of-fit statistics or results of goodness-of-fit testing (including if relevant a rationale for selection/use of a particular function) that might be available for the assay and/or its prediction (e.g. r2, r2 adjusted, standard error, sensitivity, specificity, false negative and false positive rates, predictive values, etc). There are multiple appropriate ways to analyse the large number of concentration curves coming from HT screening assays, and there is no single "right way." Therefore, in addition to specifying the fit, it is equally important to explain the curve fitting process, the assumptions used to determine the goodness-of-fit and any limitations related to the data analysis. Indicate whether a cross-validation was carried out and statistics used (e.g. leave-one-out or other statistics).

4.4 Scope and limitations of the assay, if known: Describe the types of substances in terms of their physical properties or similar (e.g. specific types of substances) for which the assay is appropriate. If possible characterise the applicability domain systematically using physicochemical and other relevant molecular descriptors using the training sets of reference chemicals that were used during the development or validation of the assay itself. Descriptions should include physical-chemical limitations and discussion of the chemical space (i.e. known applicability domain as regards key physical-chemical properties and other molecular descriptors, chemical class(es)) that has been covered in the development of the assay(s)

and use of assay results in the prediction model. Is the test known to be amenable/not amenable to a variety of chemicals such as mixtures, UVCBs, multi-constituent substances, organometalics, inorganic substances, discrete organic substances and various chemical classes or organic substances? Indications from the false positives/false negatives identified that the assay has specific limitations?

Describe for example the inclusion and/or exclusion rules that may also define the scope of the assay, e.g. substances with high volatility, hydrophobicity or lipophilicity. Limits of applicability could also include established limitations of the assay, e.g. for the Bovine Corneal Opacity and Permeability assay, high false positive rates are known for alcohols and ketones and high negative rates have been established for solids; e.g. UV absorbance of some compounds or highly coloured substances or autofluoresence could interfere with spectrophotometric analysis.

5. Potential Regulatory applications

This section provides information to help build a contextual weight of evidence analysis on the (quantitative) use of the prediction model for different regulatory purposes indicating all its potential applications.

5.1 Context of use: Propose possible conditions of use referring to effects investigated and the AOP if possible. This section is aimed at describing the level of scientific confidence for different end use scenarios i.e. where there is adequate scientific confidence for the use of a given prediction model and the rationale for this. Examples of end use scenarios could include, but are not limited to:

- support category formation and read-across: The outcomes from the assay could be used to substantiate a hypothesis for grouping substances together for the purposes of read-across;
- Priority setting: The assay might help prioritise substances within an inventory for more detailed evaluation;
- Screening level assessment of a biomarker or mechanistic activity or response: The assay might provide a reasonable indicator of the likely outcome in an in vivo assay. The screening level assessment may even be sufficient to identify a hazard, inform classification & labelling or even potency;
- Integrated approaches to testing and assessment (IATA): The assay may form one component of an IATA;

6. Bibliography

Report useful references other than those directly associated with the assay or prediction development (references describing the model development are reported in field 1.8).

7. Supporting information

Indicate whether supporting information is attached (e.g. external documents) to this MD and specify its content and possibly its utility unless previously annotated in the preceding sections.

ABBREVIATIONS

AOP: adverse outcome pathway

MD: Method description

DB-ALM: Joint Research Centre's (JRC) DataBase service on ALternative Methods to animal experimentaion (Last access: 21.02.2014 http://ecvam-dbalm.jrc.ec.europa.eu/)

ER: estrogen receptor
HTS: High Throughput Screening

HCS: High Content Screening

IATA: Integrated Approaches to Testing and Assessment

IOM: Institute of Medicine

OHT201: OECD Harmonised Template 201 (harmonized template for key events)

MIE: Molecular Initiating Event