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F.3 Chemical Safety and Alternative Methods
European Union Reference Laboratory for Alternatives to animal testing (EURL ECVAM)

Summary Record

EU-NETVAL Meeting 26th-27th November 2015, Ispra, Italy

The second meeting of EU-NETVAL was held on 26th- 27th November 2015 (the agenda is included in Annex I).

EURL ECVAM provided a brief overview of the work done by EURL ECVAM and updates. This included an overview of the validation workflow, with an emphasis on the coordination of validation, stakeholder engagement, regulatory acceptance and international recognition and dissemination. The regulatory context is fundamental and the focus here is on the **3Ps**: while the **principles** of validation remain scientifically grounded and relatively constant, the **purpose** and **process** of validation are evolving to keep pace with scientific progress and address the needs of decision makers.

The EURL ECVAM Status Report was published on 14 October 2015 and describes ongoing research and development activities, validation studies, peer reviews, EURL ECVAM Recommendations, EURL ECVAM strategies and international acceptance of alternative methods and approaches.

The emerging concept of the IATA (Integrated Approaches to Testing and Assessment) was introduced, and this was covered in more detail on the second day of the meeting. EURL ECVAM detailed its significant contribution to the OECD AOP development program, especially its active participation in (and co-chairing of, on behalf of the EU/EC) the Extended Advisory Group on Molecular Screening and Toxicogenomics (EAGMST) and its role in building the Adverse Outcome Pathways Knowledge Base (AOP-KB). The major research initiative SEURAT-1 held a final symposium in December 2015 and the 5th Annual Book of SEURAT-1 was launched at EUROTOX 2015 in Porto, Portugal. Copies of the book in electronic or printed form are available from www.seurat-1.eu.

Other recent activities include EURL ECVAM's contribution to the Commission's Communication (led by DG ENV) in response to the European Citizens' Initiative "Stop Vivisection". EURL ECVAM are leading Action 1[1] which includes a public survey, the aim of which is to solicit input from individuals and organisations i) to identify all types of knowledge sources that might be relevant to Replace, Reduce or Refine (the '3Rs') the use of animals for scientific purposes ii) to understand how such knowledge is currently disseminated and iii) to highlight what could be done to fill knowledge gaps and enhance knowledge sharing. The outcome of the survey will be made public by the end of 2016.

Further updates from EURL ECVAM covered hosting the first meeting of European 3Rs Centres to explore common interests; a visit of a Brazilian government delegation to discuss joint actions for cooperation on alternative methods; the meeting of the International Cooperation of Alternative

[1] Action 1 - Building on existing activities of the Commission, relevant EU agencies and OECD, the Commission will analyse technologies, information sources and networks from all relevant sectors with potential impact on the advancement of the Three Rs, and will present by end 2016 an assessment of options to enhance knowledge sharing among all relevant parties. The assessment will consider how to systematically accelerate knowledge exchange through communication, dissemination, education and training.

Test Methods (ICATM) and the bilateral meeting with IVTIP (*In Vitro* Testing Industrial Platform). This latter meeting highlighted the importance of regulatory acceptance from a commercial perspective as well as matching *in vitro* R&D with regulatory testing requirements and expediting the regulatory acceptance of "me-too" methods. EURL ECVAM is currently developing a guidance document on validation of *in vitro* methods which is intended to serve as a practical resource primarily for test developers interested in undertaking a validation study to demonstrate the reliability and/or relevance of their method.

Review of EU-NETVAL terms of reference

EURL ECVAM presented an overview of EU-NETVAL and the Terms of Reference. The recent call has resulted in the expansion of the network to include 13 new test facilities (bringing the total to 37). Fifteen countries are now represented in the network (from EU MS and EFSA countries).

The question of how EU-NETVAL is financed was raised during the meeting. In particular, how the individual Member States (MS) support the reference laboratories. The basis for the support from MS is in Article 47 of Directive 2010/63/EU[2], but the nature of this is not defined and is open to interpretation. Consequently, the contributions from each MS differ. Promoting alternative approaches and contributing to the development and validation of alternative approaches are different things and this also needs to be clarified within the Terms of Reference.

Currently, there is a facility on the DG for the Environment website for MS to provide voluntary reports on how they are supporting the development, validation and promotion of alternative approaches at the national level. Here, MS can share their progress and also good practice which may act as a means of encouraging others to contribute. Again, the level of contribution may not be clear.

- EU-NETVAL members were encouraged to contact their NCPs to inform themselves about opportunities for financing validation activities. Members were also urged to get to know the PARERE members for their respective MS.
- The legal infrastructure and the financing of EU-NETVAL need to be addressed in order to maximise the potential of this network. In the coming years, it is anticipated that the activities of the network will be increased.

EURL ECVAM activities directly relevant to EU-NETVAL

Progress report on the AR-CALUX validation study

EURL ECVAM gave an overview of the study from the selection of the participating laboratories to the selection of the test chemicals. Following consultation with the Validation Management Group for Non-Animal Testing (VMG-NA), chemicals with non-specific response and dual response are going to be included in the chemical list. The training for this study took place in February 2015 and there is a technical report detailing the experimental part of this training available on CIRCABC[3]. The transfer phase has started and is expected to be finalised at the beginning of 2016. The following phases (study 2 and study 3) will be initiated in 2016. EURL ECVAM will strive to draft the validation study report in 2017 and present it to the ESAC peer review panel as well as to the OECD VMG-NA. The WNT will then review it. This validation study will also work towards inclusion of the validated method in the performance based test guideline (PBTG) on androgen receptor transactivation assays (ARTA) (all mechanistically similar methods). At least two methods must be covered under a PBTG. Currently there is the AR-STTA with AR-EcoScreen cells (Japan); AR-STTA with 22Rv1/MMTV (GR-)

[2] DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010
[3] CIRCABC - Communication and Information Resource Centre for Administrations, Businesses and Citizens

human prostate cancer cell line (Korea) and possible the YAS assay wtih *Saccharomices cerevisea* (yeast) cells (BASF).

The three participating Test Facilities (TF) CiToxLab (France), Envigo UK (formerly Huntingdon Life Sciences) and SP Technical Research Institute (Sweden) provided their feedback on the usage of the method to be validated. All reported that the training was very useful and enjoyable and it was very important to see the manual procedure. Updates on their respective progress were also given. Difficulties encountered included the differences in the equipment that each TF uses, illustrating why the transfer phase of the validation study is crucial.

Design of a protocol to determine the specificity of an antagonist response in a transactivation (gene-reporter) assay

A decreasing response in an antagonist assay can be due to true antagonism, or due to suffering of the cells (cytotoxicity) or other type of interference with luciferase production. Therefore, a specificity control is essential in this assay to ensure true antagonism. The preliminary findings of a study to examine the effect of different concentrations of dihydrotestosterone (DHT) (other than the EC_{50}) in the assay medium used in the antagonistic protocol were presented by EURL ECVAM. The study design and the 11 chemicals tested were described and it was explained how criteria were being investigated and developed to define true from false responses. The preliminary findings noted an interesting pattern for chemicals suspected to have dual behaviour: these may show agonistic response in the presence of low concentrations of the agonist DHT. The chemical does not decrease the response generated by agonist DHT.

Further chemicals may need to be tested. Other distance measures between specificity curve and response under the antagonist protocol may also be considered. The classifier will be proposed at the end of study 2.

Status of the EURL ECVAM project on a method and standards for *in vitro* estimation of human hepatic metabolic clearance

There are several *in vitro* methods that are used to measure *in vitro* hepatic metabolic clearance as a mean to predict *in vivo* hepatic metabolic clearance. In order to ensure that data derived from *in vitro* clearance methods is sufficiently reliable and relevant to be used in regulatory decision making, the aim of this project is to develop a validation framework that can be systematically and routinely applied to evaluate and describe existing methods and those that will be developed in the future. The process to develop this framework is based on a comprehensive set of *in vitro* standards that can be used to fully characterise and describe the most important elements of a test method, to evaluate the performance of the method, and to report all this information in a structured and easily accessible way.

The process to generate the standards involves the definition of a "representative method" which is built upon data-gathering of existing knowledge in the field of *in vitro* human hepatic metabolic clearance methods. An overview of the commonly used and currently investigated, but not yet established test systems, was presented along with the aims and the types of standards (reporting, chemical and procedural standards). A set of SOPs is currently being created for the identified "representative method".

Status of the EURL ECVAM project on a method and standards for characterisation of metabolic/biotransformation competent *in vitro* human hepatic test systems

EURL ECVAM presented the CYP induction multi-study validation trial. In 2014, ESAC encouraged EURL ECVAM to continue conducting studies with human hepatic models to develop methods for characterization of other kinetic data, including clearance, metabolic profiling and inhibition. In

2015, this was submitted as a draft OECD Performance-based Test Guideline (PBTG) on CYP induction and reviewed by the OECD expert group. This expert group considered the CYP *in vitro* test method robust and reliable and supported the submitted PBTG. According to these, a new cell line is taken for characterisation for Phase I and Phase II metabolic competence by incubating a specific probe substrate for each enzyme and measuring the metabolite formed. The rate of metabolite formation is used for the determination of the activity of each specific enzyme.

Good *In Vitro* Method Practice (GIVIMP)

EURL ECVAM outlined the purpose and scope of this document and described the draft content. Work previously carried out by four working groups did not fully address all of the identified topics. The aim of this workshop was to discuss and formulate ideas relating to Good *In vitro* Method Practice (GIVIMP). Three breakout groups each focused on a different area: 1) solubility; 2) SOPs and 3) reporting. Workshop participants addressed specific questions that had been prepared by EURL-ECVAM and a summary of the discussions which took place can be found in Annex 1.

Aspects of method validation

Method validation process

EURL ECVAM presented an update on the test submissions since 2014 and described the two-step process. Further details on these can be found in the EURL ECVAM Status Report 2015 as well as via the website. Calls are occasionally launched by EURL ECVAM to get test submissions for areas which are considered a priority. EURL ECVAM also defines strategies in different toxicological areas to address different regulatory areas, review the progress which has been made to date and to outline what actions should be taken to deliver solutions with 3Rs impact. The validation workflow process was outlined and the roles of the ESAC peer review panel, and of the other advisory networks such as PARERE, ESTAF and ICATM, were explained.

Validation in the context of Integrated Approaches to Testing and Assessment (IATA)

EURL ECVAM described the concept of the IATA and how this next generation in safety assessment can improve on conventional toxicology which uses animal models. The OECD defines an IATA thus: "a framework for hazard identification, hazard characterisation and/or safety assessment of a chemical or group of chemicals which integrates and weights all relevant existing data and guides the targeted generation of new data where required to inform regulatory decision-making regarding potential hazard and/or risk." The workflow and the elements within an IATA were described, as well as the decision making process which makes use of the Weight of Evidence (WoE) approach. A reporting template has been developed at the OECD based on 6 principles for defined approaches. There is also a template for reporting individual information sources. Both draft templates are currently under review.

Assessing reproducibility

The reliability of a test method is one of the key aspects evaluated during the validation study and is often measured as a percentage of concordant predictions (positive/negative) for a given set of test items. Although this approach seems to be simple and widely accepted, the measure of reliability it provides is not precise enough and in addition to that it might not always be easy to implement. This includes situations where more data are generated (e.g. 4 labs involved instead of 3, more than 3 runs for some test items etc.) or when the prediction model is complex (e.g. based on the results from more than one experiment). Moreover this ad-hoc measure doesn't provide information about the underlying variability of the data that might be crucial when performance of an assay has to be evaluated. A new way to evaluate the reliability (and relevance) of a test method in the context of

validation studies was proposed and an illustrative example based on the h-CLAT validation study was provided.

Knowledge sharing in the context of the Commission's response to the European Citizens' Initiative "Stop Vivisection"

Directorate-General for Environment (DG-ENV) outlined the concept of the European Citizens' Initiative. "Stop Vivisection" is the third successful initiative, with 1.2 million citizens having signed the petition. This initiative calls for the abrogation of Directive 2010/63/EU and that the EC presents "a new proposal that does away with animal experimentation and instead makes compulsory the use - in biomedical and toxicological research - of data directly relevant for the human species." The Commission's response is that full replacement is the ultimate goal, but a complete ban of animal testing is premature. Both animal and non-animal models have their limitations. Better sharing of information is necessary to move forward in the area of alternative approaches and EURL ECVAM is taking a leading role in Action 1 above). The Commission, led by DG-ENV, will facilitate a scientific debate and will organise a conference at the end of 2016 (Action 4[4]).

EU-NETVAL members have a role in the promotion of alternative methods and were asked if they would agree to make their competences and training capacity known to all other EU NETVAL members or even publicly available. This can be discussed during the preparation of the EURL ECVAM report, "Opportunities to share knowledge and best practice within the EU-NETVAL network".

Sharing knowledge within EU-NETVAL through training

New methods and training needs and capacities

EURL ECVAM presented an overview of the initial responses to the EU-NETVAL survey on training. The main points are summarised here, but for further detail, please see the EURL ECVAM technical report, "Opportunities to share knowledge and best practice within the EU-NETVAL network".

Whilst the primary beneficiaries are EU-NETVAL members, (the aim is to promote knowledge sharing and training collaboration), there is also the possibility to contribute to Action 1 above) of the Commission Communication in response to the European Citizens' Initiative "Stop Vivisection", which aims to promote better knowledge sharing. The EU-NETVAL survey aims to identify opportunities to share knowledge and best practice within the EU-NETVAL network by:

i) mapping the methods being implemented by NETVAL members and the associated competences,

ii) identifying particular training needs of members, and

iii) gathering ideas on how to best share knowledge through training initiatives.

In the outline of the workshop, EU-NETVAL members were asked to draw on their own experiences and ideas for using the network effectively to accelerate progress in the development of alternative approaches. A summary of the discussions that took place can be found in the EURL ECVAM technical report, "Opportunities to share knowledge and best practice within the EU-NETVAL network".

[4] Action 4 – To facilitate an efficient dialogue, by end 2016 the Commission will organise a conference engaging the scientific community and relevant stakeholders in a debate on how to exploit the advances in science for the development of scientifically valid non-animal approaches and advance towards the goal of phasing out animal testing.
On that occasion, the Commission will also report progress on actions 1, 2 and 3.

Promising assays: outcomes of EU-NETVAL survey

This survey may also help in identifying promising methods being used by EU-NETVAL members. EURL ECVAM hopes to find good candidates for validation, as indicated for example in EURL ECVAM strategies that aim to achieve 3Rs impact in different areas of regulatory safety assessment.

The discussion which took place during this session indicated that clients often request modified versions of TG methods for a specific purpose. EURL ECVAM is particularly interested in learning about any non-guideline methods which are being routinely requested by clients as potential methods for validation. EU-NETVAL members were asked to please contact EURL ECVAM if and when they have any information related to this.

The initial response to part one of the survey was presented in order to give the network an idea of some of the competences that exist within it. The survey was reopened for the new members and the results will be published in the EU-NETVAL technical report, "Opportunities to share knowledge and best practice within the EU-NETVAL network" which will be circulated for comments before being made publically available. The published version of the report will respect the privacy of the individual test facilities and will therefore be a profile of the network.

CIRCABC

CIRCABC is the platform used for sharing documents amongst network members. Documents and presentations for meetings are uploaded here. A brief guide on how to use CIRCABC was presented and is available from EURL ECVAM if needed. It was mentioned that we could use the 'newsgroup' feature to create a discussion forum and this is an option that EURL ECVAM can look into.

Follow-up discussions on topics raised by partners

- *The financing of EU-NETVAL activities may present a challenge for some facilities, particularly when it comes to attending meetings. For this reason, there will be one meeting per year. It was suggested that part of the meeting could be dedicated to technical aspects relating to methods.*
- *The meetings could be hosted by other members (not only the coordinator, EURL ECVAM). EURL ECVAM could assist with the financing for another facility to host the meeting. This is open for further discussion.*
- *All members were reminded to notify EURL ECVAM if there are any changes in their organisations/companies.*

Annex 1

GIVIMP Breakout groups: Summary of discussions

Three breakout groups each focused on a different area: 1) solubility; 2) SOPs and 3) reporting. The orientation for the workshop may be found in Annex I.

Solubility

A number of questions (with preliminary answers) on solubility issues were made available to NETVAL via the OECD, for consideration and development in GIVIMP. The discussion was introduced as a NETVAL meeting breakout group (BoG) with expectation for follow-up by a focused working group. With only 2 hours allocated to the BoG (including reporting back to the plenary meeting) it was decided to limit the discussion to 4 principal questions.

First, as an introduction (and for discussion reference) 3 examples of ECVAM experience in solubility testing were given, implemented in practice during validation studies, indicating evolving scope and refinement:

1) 2010: hCLAT (skin sensitization) (24 chemicals): solubility determination limited to stock solutions, by visual inspection only.
2) 2011/12: CYP induction (13 chemicals): solubility determined for stock solutions and medium dilutions, also with stability monitoring during incubation, but by visual inspection only (according to SOPs available from method developers). Application of nephelometry (EURL-ECVAM in-house only) was also developed during this validation study, with systematic testing of the 13 chemicals.
3) 2014/15: AR-CALUX (75 shortlisted chemicals): Nephelometry established at EURL-ECVAM (with SOPs for test item preparation, instrument operation and data evaluation) applied to the 75 chemicals (EURL-ECVAM in-house only).

With the limited time available, discussion focused mainly on the first of the 4 questions:

Q1. Which methods are practical for solubility determination?

An overview of familiar analytical methods was presented for comment, i.e., visual inspection, nephelometry, and HPLC/UV spectrophotometry, noting the following:

- Visual inspection: simple, but also subjective and imprecise.
- Nephelometry: systematic, rapid, and sensitive, but dependent on instrument availability

  and definition of turbidity threshold for insolubility.
- HPLC/UV spectrophotometry: both provide quantitative analysis of concentration, but unsuitable for complex matrices (e.g., cell culture incubation media).

Possibly, GIVIMP could include a tiered approach, depending on application and relevance.

Comments on methods:

- Distinction of 'thermodynamic' versus 'kinetic' solubility: essentially, 'thermodynamic' means equilibrium concentration (with excess solid) while 'kinetic' means metastable concentration (when induced precipitation first occurs, e.g., by adding the chemical as stock solution to assay medium, including incubation). The latter is generally relevant to *in vitro* method applications.

- Visual inspection is generally sufficient for simple checking of solubility where reliability can be enhanced by use of microscopy to detect solid particulate or liquid droplet suspension (indicative of insolubility). Reliability also improved by centrifugation, particularly for detection of precipitation in medium dilutions, where foaming may obscure visual observation.

- Nephelometry (less familiar) is particularly suited to stability determination and monitoring in assay medium (e.g., as serial batches of chemicals and/or concentrations) allowing precise critical detection of turbidity due to precipitation, independent of matrix composition. However, setting a turbidity threshold for definition of insolubility is arbitrary and dependent on sensitivity (i.e., relative to a designated standard turbidity and/or resolution of significant signal from background). Moreover, investment in nephelometry would only be justified by requirement of routine high sample throughput (e.g., comparison of numerous chemicals and/or concentrations under specific conditions).

- HPLC and UV spectrophotometry provide quantitative determination of concentration via standard curve calibration: HPLC by eluent peak area, UV spectrophotometry by wavelength absorbance. The latter, in principle involving only plate reader measurement without chromatographic species separation, would be the more efficient. However, while both methods are valid for stock solutions in solvent, application to biological media would introduce interference of matrix composition. Culture incubation media could not be injected into HPLC columns, and anyway, would likely obscure determination of the solute chemical by inherent UV absorbance over a range of wavelength.

Comments on procedure (sample preparation): The group considered the following issues, but without conclusion:

- Time for dissolution in solvent (immediate vortex only versus overnight standing)?
- Conditions for medium incubation stability: general guidance or assay dependent?
- Molarity (µM) versus gravimetric (µg/mL) basis for setting concentrations?

Q2. What relevance has solubility (i.e., starting concentration) to dose-response, etc.?

The following issues were raised for comment, and although inconclusive, were considered relevant for further discussion and possible inclusion in GIVIMP:

- Influence on reproducibility/predictive capacity of assay (within and between labs)?
- Influence on dose-response, e.g., EC50, IC50 determination?
- Influence on cytotoxicity assessment, etc.?

- Other issues?

Q3. What GIVIMP (guidance) for top concentration (otherwise arbitrary or assay dependent)?

The question concerns whether guidance is relevant to setting a target concentration (e.g., 50mg/mL or 100mM) for stock solutions in solvent, with 1000X dilution in medium. The ideal might otherwise be to seek the true maximum soluble concentration (at thermodynamic saturation and equilibrium). The discussion was introduced noting previous examples (EURL-ECVAM validation studies):

2010: hCLAT (skin sensitization) (24 chemicals):

- Starting concentrations: 100mg/mL (saline) or 500mg/mL (DMSO):

Comment: these concentrations seem rather high, but are now included in a draft OECD TG.

2011/12: CYP induction (13 chemicals):

- Starting concentrations: 40mg/mL (i.e., 100mM for MW=400).

2014/15: AR-CALUX (75 shortlisted chemicals):

- 3 trial concentrations prescribed for solubility testing to determine maximum compatible: 50, 15, 5 (mg/mL: stock solutions; µg/mL: medium dilutions) (abbreviated: C50, C15, C5).

General Comments:

- maximum soluble concentration would be laborious to determine, particularly for multiple chemicals (and different incubation media).
- "conventional" target (top) concentrations may be unrealistically high?
- top concentration may be assay dependent (possibly up to the method developer to verify) also with consideration of solvent interference.

Q4. What GIVIMP (guidance) for dilution steps, if a chemical is insoluble in medium at a preferred concentration (e.g. 50 µg/mL or 100 µM) and is there a lower limit below which the test is no longer applicable or relevant?

Discussion here was again introduced with reference to previous examples (EURL-ECVAM validation studies):

- CYP induction: dilution by halves (2-fold):
  - CryoHep: 100, 50, 25, 12.5, 6.25 mM (for stock solutions) +1000-fold in medium.
  - HepaRG: 40, 20, 10, 5 mg/mL (for stock solutions) +1000-fold in medium.
- AR-CALUX: dilution by thirds (3.33 / 3-fold): C50, C15, C5.

Comment/Question: if the chemical is insoluble at C5 (stock solution or medium dilution) should it be considered effectively incompatible with the assay?

General comment:

- probably a case-by-case issue, dependent on assay, to achieve a relevant dose-response.

SOPs

A brief introduction concerning the use of Standard Operating Procedures (SOPs) when developing a new *in vitro* method was given. Four areas were identified for discussion, what approach should be taken when developing *in vitro* method SOP(s), the level of detail that should be provided in the SOP(s) with regards to materials and apparatus, what aspects of the *in vitro* method SOP(s) require acceptance and performance criteria, and quality controls and how should raw data be defined in the SOP(s) so as to facilitate data analysis. A general discussion regarding *in vitro* method SOP(s) specifically in a GLP environment also took place.

The general consensus was that SOPs should be specific and not try to do too much, i.e. have shorter SOPs rather than a single (God) SOP and make a clear distinction between study design (which should be placed in the Study Plan) and the actual method SOP. It was recommended that a formal validation of the SOP should be performed in-house prior to use in a GLP environment. It was recommended to state in the SOP if it requires in-house validation prior to use.

With reference to the amount of detail provided in the SOP it was recommended that the use of catalogue numbers should only be used when critical to the SOP and a better approach is to use CAS number, purity or other identifiers. This approach was also recommended as in a GLP environment there is the need to validate the supplier. Limitations of the SOP should also be clearly described in the SOP and critical steps that may require additional quality controls or quality assurance should also be identified.

With regards to equipment it was recommended that equipment specifications (e.g. sensitivity) are a better approach than specifying that actual equipment model, and again limitations should be defined. Acceptance and performance criteria, and quality controls should be applied, but not limited to the test system (e.g. positive and negative controls) and also include periodic quality controls e.g. mycoplasma, karyotyping, cell doubling time, etc. Monitoring of the reference item should also be performed.

The use of Excel spreadsheets or other software was also discussed in relation to the SOP(s) and it was recommend that a SOP on how to use these should also be provide, including how to validate (in-house) these software for use in a GLP environment.

Further points in relation to versioning of SOPs, developing SOPs in a GLP environment (difficult and more time consuming), the usefulness of data generated with a non-approved SOP were discussed.

Reporting

References for this topic:

1. OECD principles on good laboratory practice number 1 and 14.
2. OECD series on Testing and Assessment, number 211 for describing non-guideline *in vitro* test methods (referring to OHT 201).
3. Draft OECD harmonised template 201 (OHT 201) for the reporting of intermediate effects.
4. ECHA practical guide on how to report *in vitro* data in IUCLID 5 (using OHT 201).
5. Guidance on good cell culture practice, ATLA 33, 261–287, 2005.
6. ISA Tab; http://isatab.sourceforge.net/index.html, a tool for description of experimental metadata.

EURL ECVAM clarified that the GIVIMP document should provide guidance for all users of *in vitro* methods; the test developer, the validation body and the regulator. By giving guidance to the test developer on what information to provide and results to be reported, the validation body and regulator will have sufficient information for decision making. The references were introduced.

The OECD harmonised template 201 (OHT201), recently developed and almost approved, is used for the reporting of intermediate effects. Any information which gives only partial information and not a full classification can be reported with this OHT201. It should be clarified if the OECD template 201 is relevant only to regulators or if it can be used by everyone.

EURL ECVAM had used the ISATAB tool for the SEURAT project to create the TOXBANK data base with the aim that any other lab can extract and reuse the data, to avoid duplication. In this way, data are harmonised and can be immediately distributed and shared. The ISA-Tab tool was briefly presented by Elisabeth Joossens.

The group thought that reporting requirements should probably be different for the different development phases of an *in vitro* method (development, pre-validation and validation) and that the elements to be reported must be led by the needs of the audience. There is a need to standardise the reporting format, depending on the phase.

Comments/ replies were received from the group to the following questions:

Q1. During the development of an *in vitro* method, what are the important meta-data that we should document and report, to give confidence in the method and help possible pre-validation? For which essential test method components do we need to have reported historical data?

A: For the essential equipment, to ensure it is calibrated and functions correctly.

A: All data used for the development and optimisation of the method are important to be reported. E.g. selection of the optimal CO_2 level in the incubator.

A: The crucial data required by the SOP should be reported, and also the operator(s) who performed the assay.

It was furthermore added that it is important to make a distinction between what information you should record (as requested by the SOP) and what information you should report. General background information must be recorded (e.g. the number of operators involved in the development, dates, relation dates/operators), but not all information must be reported. The test developer should record as much information possible during the development of the method and decide what to report later. The knowledge that the information is available on request is important.

A: For the reference and control items information such as batch to batch variation, stability and justification for selection should be reported.

A: For the Test system information such as viability, stability and integrity, demonstration of correct functioning and time/performance window should be reported. The performance of the test system must be assessed, by applying acceptance criteria, to demonstrate correct function of the test system. The parameters to monitor depend on the test system E.g. For hepatocytes the production of urine is an important function and can be used as parameter for changing the performance of hepatocyte like cells. Explaining the rationale for the acceptance criteria of the assay is important.

A: Track the important changes in the SOP, with motivation of the change(s). Where possible, data should be available to avoid repetition.

Q2. What kind of meta-data should we report in addition to the results for the test item (for (pre-validated *in vitro* methods) to interpret the results? For which essential test method components the reporting of meta-data can and should be standardised?

A: To be able to judge the robustness of the method it is important to record the number of operators that have been involved in the data production and method development. Also for the pre-validation it is important to know who has produced which data.

A: Meta-data have a specific purpose, which should be identified. Any data giving more trust to the results should be considered for reporting.

A: The equipment readout parameter (e.g. OD, RLU) must be reported plus the proof of correct functioning of the equipment (linearity, limit of detection etc.). These meta-data information should be standardised and reported.

A: For data interpretation it is important to know the carrier solvent (e.g. DMSO, methanol, water). Because this can change the results even if the percentage of solvent is low and theoretically it should not interfere with the test system. Limitations of the method must be known and we must see proof that the limitations are correctly considered.

A: For the pre-validation of an *in vitro* method the material and batch number of all material used, even the plastic ware, must be reported as this might influence the final results.

Q3. Is it necessary to provide the final results and meta-data in electronic format such as an excel template or software tool?

A: Yes it is important to aid the data interpretation and to ensure correct data transfer.

A: When the method is under development it is important to have an e-format of the results and meta-data, to aid the data interpretation and verification of correctness. When the method is validated you need confirmation of available information, but no need to report the electronic files.

Q4. Will an Excel template be sufficient for reporting results and meta-data, or is there a need for software tools such as ISA-Tab?

A: For validated methods: Depends to who you are reporting. For regulators probably OECD template OHT201 for intermediate effects. When sharing of data is desirable (unless confidentiality issues exist), the use of a software tool such as ISATAB can be useful.

Other considerations:

Data should be shared by default, unless there is a reason for confidentiality.

Consider who is the audience/recipient of the *in vitro* method. Separate recording and reporting. Not everything recorded should always be reported, but it is important that the information is gathered.

At university guidelines are rarely used, equipment is less monitored and comparison with historical data not performed. Therefore, the dissemination of the final guidance to reach this target group is very important.

All agreed that essential information should be preserved.

Annex I

EU-NETVAL Workshop on Good *In vitro* Method Practice (GIVIMP)

'Guidance document on Good In Vitro Method Practices (GIVIMP) for the development and implementation of in vitro methods for regulatory use in human safety assessment.'

Objectives of the workshop

The aim of this workshop is to discuss and formulate ideas relating to Good *In vitro* Method Practice (GIVIMP). Three breakout groups will each focus on a different area: 1) solubility; 2) SOPs and 3) reporting. Workshop participants will address specific questions and elect a rapporteur to feedback the main points in the plenary session.

Background

In vitro methods, often based on the use of human cells and tissues, are submitted to international validation bodies and/or to receiving authorities. Well-designed, robust, reliable *in vitro* methods that can run in a GLP environment for generating data sets are becoming more and more instrumental for supporting regulatory decisions. Good *In vitro* Method Practice (GIVIMP) is a proposal from EURL ECVAM to issue an international guidance for the development and implementation of *in vitro* methods for regulatory use in human safety assessment. GIVIMP will contribute to increased standardisation and harmonisation in the generation of *in vitro* information on test item safety. The Guidance will further facilitate the application of the OECD Mutual Acceptance of Data agreement for data generated by *in vitro* methods and as such contribute to avoidance of unnecessary additional testing. GIVIMP will take into account the requirements of the existing OECD guidelines and advisory documents to ensure that the guidance is complementary and 100% in line with these issued documents.

We hope that with your input the GIVIMP guidance will contribute to the increased use of *in vitro* method data to support regulatory human safety assessment of industrial and household chemicals, food additives, cosmetics, mixtures etc. by striving that such data are being generated in compliance with GLP and based on current good scientific and technical practices.

Scope and use

This guidance describes the areas related to *in vitro* method development, standardisation, harmonisation and international acceptance that would benefit from more detailed scientific, technical and quality guidance.

This guidance is not intended to duplicate or replace any OECD Guidance or Advisory documents but rather it is complementary, addresses specific gaps and aims to collect all available references and information on best scientific, technical and quality practices in one document. It aims to accelerate and reduce time (first time right) of the development of purpose-directed, high quality *in vitro* methods (forming the basis) for bioassay certification/authorisation.

This guidance document mainly targets all players involved in the process e.g. *in vitro* method developers, *in vitro* test system producers, *in vitro* method validators, *in vitro* method test guideline or performance based test guideline issuers, *in vitro* method receiving authorities and overall users.

The workshop will be divided into the following areas and address the relevant questions:

1. Solubility

Solubility testing is necessary in the characterisation and preparation of the test system. In particular, how solubility is measured is crucial to establishing the correct concentration of a substance in an assay. Achieving a common understanding and approach to solubility measurements is fundamental to good *in vitro* method practice.

2. SOPs

SOPs should be clear, brief and easy for trained personnel to follow as well as emphasizing the critical steps and warning about safety issues. It needs to be reviewed and updated systematically.

Purpose and benefits of the SOP

Facilitate consistency in quality and integrity of a product or end result

Reduce work efforts

Improve comparability, credibility and legal defensibility

Type of SOPs

Technical

Administrative

Equipment

Facility

A SOP should be dedicated to only one well defined task, and refer to another SOPs for associated tasks or important information (e.g. equipment to be used, facility requirements, calibration,…..)

3. Reporting

Capturing test observations in a coherent, widely accepted data format will facilitate comparison of the data and test methods under review.

Annex 2

Agenda - EU-NETVAL Meeting 26th-27th November 2015

Thursday 26th November 2015

Auditorium 58c
Breakout rooms 12a, 12b and 1302 (Building 101)

08:15 *Bus transfer from hotel to JRC campus*

09:00 - 10:30 **Welcome and introductory session**

- EURL ECVAM updates
- Review of EU-NETVAL terms of reference
- Recent call to expand the network

10:30 - 11:00 *Coffee break*

11:00 - 13:00 **EURL ECVAM activities directly relevant to EU-NETVAL**

Progress report on the AR-CALUX project
Anne Milcamps, EURL ECVAM

Design of a protocol to determine the specificity of an antagonist response in a transactivation (gene-reporter) assays
Roman Liska, EURL ECVAM

Status of the EURL ECVAM project on a method and standards for *in vitro* estimation of human hepatic metabolic clearance
Varvara Gouliarmou, EURL ECVAM

Status of the EURL ECVAM project on a method and standards for characterisation of metabolic/biotransformation competent *in vitro* human hepatic test systems
Chiara Zorzoli & Siegfried Morath, EURL ECVAM

13:00 - 14:00 *Lunch*

14:00 - 16:00 **Sharing knowledge within EU-NETVAL through training**
Tracey Holley, EURL ECVAM
- Summary of responses to the training survey
- Breakout groups

16:00 - 16:30 *Coffee break*

16:30 - 17:30 **Sharing knowledge (continued)**
- Reporting back of breakout groups
- Discussion

17:30 - 18:00 **Knowledge sharing in the context of the Commission's response to the European Citizens' Initiative "Stop Vivisection"**
Susanna Louhimies, DG ENV

18:00 *End of the Day*

18:15 *Bus transfer to Restaurant Conca Azzurra*

19:00 Dinner at Ristorante Conca Azzurra, Via Alberto, 53, 21020 Ranco

21:30 Bus transfer from Ranco to hotel

Friday 27th November 2015

Building 100 Room Acqua
Breakout rooms 12a, 12b and 1302 (Building 101)

08:15 *Bus transfer from hotel to JRC*

09:00 – 11:00 **Good *In Vitro* Method Practice (GIVIMP)**
Ann-Charlotte Bostroem, EURL ECVAM
- Introduction and status
- Breakout groups
- Reporting back and discussion

11:00 – 11:30 *Coffee break*

11:30 - 13:00 **Aspects of method validation**

Method validation process
Valérie Zuang, EURL ECVAM

Validation in the context of IATA
João Barroso, EURL ECVAM

Assessing reproducibility
Roman Liska, EURL ECVAM

13:00 - 14:00 *Lunch*

14:00 - 15:00 **Promising assays: outcomes of EU-NETVAL survey**
Summary of responses to survey on methods implemented by facilities
Ann-Charlotte Bostroem, EURL ECVAM
- Discussion

15:00 - 16:00 **Follow-up discussions on topics raised by partners and wrap-up**

16:00 *End of the Meeting*

Transports to the airport