EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Health, Consumers and Reference Materials
Chemical Safety and Alternative Methods Unit
EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM)

Summary Record

EU-NETVAL Meeting 10-11th October 2016, Ispra, Italy

Welcome and Introductory Session

EURL ECVAM welcomed the member facilities to the JRC, highlighting the policy focus of the work which the Chemical Safety and Alternative Methods unit carries out. This includes the regulation of chemicals, advancing animal-free methodology for improved risk and hazard assessment whilst facilitating innovation and trade to strengthen the EU industrial base. EURL ECVAM's responsibilities were established under Directive 2010/63 on the protection of animals used for scientific purposes. As such, these responsibilities include: guiding research on alternative methods; coordinating validation within the EU, dissemination of information on the 3Rs; facilitating stakeholder dialogue; and promoting international acceptance of alternative methods.

As part of its extensive activities to drive progress in the area of alternatives, EURL ECVAM works with a wide range of stakeholders and scientific advisors as well as numerous international collaborations, including research consortia such as EU-ToxRisk, a European flagship programme funded by Horizon 2020 which aims to deliver reliable new non-animal testing strategies for risk and hazard assessment of chemicals. It started in January 2016 and will run for six years. EURL ECVAM is also working in partnership with 3Rs centres across Europe to identify ways in which the number of animals used for biomedical research (BMR) could be reduced.

Three of the Adverse Outcome Pathways (AOPs) published during 2016 by the OECD have been developed by the Joint Research Centre's (JRC) Chemical Safety and Alternative Methods Unit. These AOPs relate to chemical-induced liver fibrosis, aspects of neurotoxicity in adults, and certain neurotoxicological effects that can be caused during human development.

A further highlight from EURL ECVAM was the publication of the new book, "Validation of Alternative Methods for Toxicity Testing", which provides an overview of the state of the art in method validation.

There has been significant progress for skin sensitisation and EURL ECVAM has provided major contributions to the updating of **REACH annexes VII and VIII** to reflect scientific progress in the areas of skin corrosion/irritation; serious eye damage/eye irritation; skin sensitisation and acute systemic toxicity. In the area of skin sensitisation, these revised provisions makes information from the newly adopted non-animal methods (Direct Peptide Reactivity Assay (**DPRA**), OECD, 2015a; **KeratinoSens™**, OECD, 2015b and the human Cell Line Activation Test (**h-CLAT**), OECD, 2016c) the default requirement. This has also prompted an update of the European Chemical Agency's (ECHA) guidance for industry, which EURL ECVAM is supporting. EURL ECVAM has also led an Organisation for Economic Co-operation and Development (OECD) project to develop two Guidance Documents on the Reporting of Defined Approaches to be Used Within Integrated Approaches to Testing and Assessment (OECD, October 2016). These documents are intended to provide guidance on how individual information sources and defined approaches (DA) developed for **skin sensitisation assessment** should be reported in a harmonised way.

Integrated Approaches to Testing and Assessment (IATA) are also key to developing alternative solutions for combined exposure and mixtures, where *in vitro* and *in silico* approaches could perhaps be considered to be the best ways to tackle these challenges. The development of IATAs will require a concerted effort from many partners.

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 has led Action 1[1] which includes the mapping of knowledge sources relevant to the 3Rs to highlight where potential knowledge gaps exist and how knowledge sharing can be enhanced. The outcome of this study was presented at the European Commission conference "Non-animal approaches: The way forward", in December 2016 and has now been published in a JRC report, Accelerating progress in the Replacement, Reduction and Refinement of animal testing through better knowledge sharing.

Updates on the current state of the network

The current state of the network was described and the tasks of EU-NETVAL members, as specified in the Terms of Reference, were outlined. The network obtained 13 new members from 3 new countries (Switzerland, Poland and Slovakia) since the beginning of 2016.

EURL ECVAM collated the information from the EU-NETVAL training and competencies survey, which was conducted during the beginning of 2016, and presented an overview of the network as well as proposals for how the knowledge within this highly competent network could be shared. This has been shared with EU-NETVAL and members have had the opportunity to provide their comments on this document. The majority of the network respondents expressed a willingness to participate in collaborative training ventures and hands-on training was deemed to be the best approach to training on *in vitro* methods. However, there are physical, financial and human limitations for training, so the emphasis must be placed on mutual benefit for both the trainer and the trainee. As a follow-up to this exercise, this year's meeting included a pilot project for training which brought EU-NETVAL members and *in vitro* method developers together to share their knowledge specifically in the area of methods for testing the skin sensitisation potential of chemicals: these included the DPRA, KeratinoSens™/LuSens and h-CLAT OECD TG methods. This marks the first of what will hopefully be many more collaborations within the network to share expertise and training to validate alternative *in vitro* methods and promote their use.

Validation workflow

Since the last meeting, there have been **six test submissions** which include 3 pre-submissions and 3 full submissions. The ESAC peer reviews which were completed in 2016 covering the endpoints of eye damage/irritation, skin irritation and skin sensitisation were described. EURL ECVAM is also preparing Recommendations on reconstructed human cornea-like based test methods (EpiOcular EIT and SkinEthic HCE), human-based CYP induction assays, and skin sensitisation. Full details of all of these are available in the EURL ECVAM Status Report which was published in October 2016.

Three pre-submissions and three full submissions were received in 2016 and the AR-CALUX validation study is ongoing.

An overview of the International Cooperation on Alternative Test Methods (ICATM) Workshop in October 2016 on the international regulatory applicability and acceptance of alternative approaches

[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.

to skin sensitisation was given. The engagement of the regulatory community and scientists from a range of countries and sectors was very positive. The outcome of this workshop will be presented in two peer reviewed publications.

The meeting of European 3Rs centres, which took place in May 2016, highlighted the specific strengths and priorities of each of the centres and explored ways in which the network could be strengthened to amplify 3Rs impact in many key areas.

AR-CALUX validation project update

Three EU-NETVAL laboratories are involved in the validation of the AR-CALUX bioassay, which is a transactivation assay to measure the endocrine disrupting activity of chemicals. The aim of the project is to develop a Performance Based Test Guideline (PBTG) with Performance Standards (PS). A PBTG already exists for Estrogen Receptor Transactivation Assays (ERTA - TG455), which describes mechanistically and functionally similar test methods for the identification of estrogen receptor agonists and antagonists, and may be used to facilitate the development of new similar or modified test methods. A similar approach is being taken for androgens with more than one method being included in the PBTG, including the validated EcoScreen assay (see OECD TG458).

Study 1, which is the transfer phase, was initiated in 2015. This phase has been very useful to understand where the SOP needed to be refined. After the transfer, the three laboratories perform studies 2 and 3 independently. The goal is to finalise all practical work by end of 2017, which will then be followed by ESAC and VMG-NA consultations prior to submitting a PBTG proposal to WNT at the OECD.

Open issues relating to Good In Vitro Method Practice

EURL ECVAM detailed the current situation relating to the drafting of a 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, which had been identified as a high priority requirement by the OECD. The aim is to reduce the uncertainties in cell and tissue-based *in vitro* methods derived predictions by applying all necessary good scientific, technical and quality practices from *in vitro* method development to *in vitro* method implementation for regulatory use. Outstanding issues which were covered by the breakout groups during the meeting were outlined: 1) Test System & Related Discussions, 2) Apparatus, Materials & Reagents; 3) Acceptance Criteria & Data Requirements.

Organisation for Economic Cooperation and Development (OECD)

The OECD presented an overview of the OECD Test Guidelines (TG) Programme and procedures for TG development. New and updated TGs are developed to meet regulatory needs of member countries, reflect scientific progress and to address animal welfare aspects and improve the cost-effectiveness of test methods. The OECD Principles of Good Laboratory Practice (GLP) and TGs are an integral part of the Council Decision on Mutual Acceptance of Data (MAD). The GLP Principles complement the OECD Test Guidelines (TG) by setting quality standards for the organisation and management of test facilities and for performing and reporting studies.

Proposals for new or updating existing TGs can be submitted using the Standard Proposal Submission Form (SPSF). TGs provide a rationale for the assay, including the applicability domain, offering guidance instead of a detailed protocol.

Highlights on current projects in the Test Guidelines work plan are the development of *in vitro* methods for skin sensitisation include U-SENS™, IL8Luc, GARD, LuSens and SENS-IS. These represent

methods for skin sensitisation Key Events 2 and 3. Further details on the current work programme can be found here.

European Commission Directorate General Environment

EU-NETVAL was established under the legal framework of Directive 2010/63/EU on the protection of animals used for scientific purposes. The Directive is firmly based on the principle of the Three Rs, Replacement, Reduction and Refinement of the use of animals for scientific purposes, extending Refinement also to the areas of breeding, accommodation and care of animals. There is a clear legal obligation to apply the Three Rs in all interaction with animals and use alternatives where available. Whilst the ultimate goal is to replace the use of animals, the Directive recognises that animal testing is still required in Europe and so their intrinsic value needs to be respected. Both the Commission and Member States have an obligation to promote alternative approaches. Member States are to *inter alia* assist the Commission in identifying and nominating suitable specialised and qualified laboratories (EU-NETVAL) to carry out validation studies. EU-NETVAL is a relatively new structure that has been established with the aim to bring more resources for the validation of alternative approaches. EU-NETVAL has a key role to play in bringing alternative methods from the test developer to the test performer. This process requires seamless collaboration between multiple players and efficient exchange of information is essential.

New *in vitro* methods with validation potential

Identifying promising *in vitro* methods

EURL ECVAM outlined the current test method submission process, which follows two mandatory steps, (presubmission and complete submission). This approach mainly fits test methods which have a regulatory application. However, with this approach it is becoming increasingly difficult to assess regulatory relevance of mechanistic methods one by one in the absence of an overarching framework and/or of a toolbox of methods to be assessed simultaneously. The current process is also not particularly well suited to identify promising regulatory methods at an early stage of development/standardisation, and test systems/methods for use in biomedical research (e.g. disease models). Therefore, several ideas have been suggested and these include more effective direct engagement with external laboratories which may be developing or working with improved or innovative methods, better engagement with research consortia as well as strengthening the established network with 3Rs centres. Systematic surveys of the scientific literature could also assist in the identification of promising new methods. A new submission portal is being considered which could be designed to fit both regulatory test methods and test methods/systems relevant for biomedical research. This would have the added advantages of surveying identified priority areas and supporting standardisation prior to entering validation. EU-NETVAL's engagement in this would be extremely valuable and members are encouraged to send their feedback and ideas regarding this.

Characterisation and description of *in vitro* hepatic metabolic clearance methods

There is increasing demand (from researchers, test method developers, regulators, end-users,) to integrate kinetics information in chemical risk assessment. This is based on the fact that exposure to a chemical does not automatically mean that all of the dose will be bioavailable and therefore able to cause a specific toxicity. Hence the knowledge of the chemical's human kinetics can assist to better design toxicity tests (both *in vivo* and *in vitro*) and interpret toxicological findings.

Kinetics (which is the results of four processes: Absorption, Distribution, Metabolism, Excretion, ADME) information is still mainly obtained from *in vivo* tests. However, since modern toxicology is relying more and more on data generated with alternative to animal testing methods, there is increasing demand to obtain information on kinetics (ADME parameters) by using *in vitro* methods

(see EURL-ECVAM TK Strategy, 2015). Since metabolism plays a key role in determining kinetics, there are already several non-guideline *in vitro* methods to measure *in vitro* hepatic metabolic clearance which can significantly vary for the experimental-settings, stage of development, intended use, reliability, relevance, etc.

With a view to enhancing the use of human *in vitro* methods for hepatic metabolic clearance in chemical hazard and risk assessment, an OECD Guidance Document (GD) will be developed in order to characterise *in vitro* hepatic metabolic clearance methods and to facilitate method comparison and the evaluation of their performance. The Guidance Document will therefore aid clearance method developers and end-user to characterise and report the salient attributes of different methods to facilitate method comparison and increase confidence in their use to support chemical risk assessment.

EU-NETVAL will be asked to review and check the guidance document and perform experimental activities to implement/validate aspects of the guidance if deemed necessary in the future.

Thyroid hormone related *in vitro* methods

Thyroid hormones (TH) are involved in numerous physiological processes, such as regulation of metabolism, bone remodelling, cardiac function and brain development. Maintenance of normal thyroid function is essential for the psychological and physiological well-being and so thyroid-disrupting chemicals may result in reductions of serum TH levels which may have significant consequences for public health. The OECD has considerably invested in the development of test methodologies and testing strategies related to Endocrine Disruption (ED) and an OECD Expert Group was tasked with elucidating the pathways linked to disruption of TH and identifying the most promising assays capable of probing various Modes of Action (MoAs) that could be brought forward for validation.

EURL ECVAM will launch an EU-NETVAL formal call to participate in the implementation of *in vitro* methods to measure TH signalling disruption. This initiative is part of an international collaborative effort, involving EURL ECVAM and the United States Environmental Protection Agency (US-EPA), with the agreement to complement each other's' activities aimed at identifying the most promising and scientifically relevant assays capable of probing various modes of action of TH disruption, in line with an Integrated Approaches to Testing and Assessment (IATA) framework, and identify which *in vitro* methods could be brought forward for validation. This is the first step towards the development of OECD Test Guideline(s) and associated Performance Standards for *in vitro* methods suitable for the detection of compounds with TH disruption potential, which are currently lacking.

New reporting approach for *in vitro* methods

The OECD Harmonised Templates (OHTs) are standard data formats for reporting studies done on chemicals to determine their properties or effects on human health and the environment. OHTs prescribe the formats by which information can be entered into and maintained in a database. By using these OHTs, governments and industry are able to electronically exchange test study summary information. The OHTs are implemented in IUCLID and are the required reporting format in several regulatory programs (e.g. REACH in the EU). The OECD invited the JRC to develop a new OHT which would allow for the reporting of observations from mechanistic (*in vitro* and *in silico*) tests which are not immediately linked to an adverse outcome (AO). The resulting OECD harmonised template 201 (OHT201) is the template to be used to report any results that are considered to be 'intermediate effects' and do not lead to a full classification of a chemical. All of the study results must be linked to a certain chemical. Reporting of data from new methods can also possibly be done with OHT 201. The bridge between scientific and regulatory communities must be crossed. If in an early stage the data can be reported this will help the regulators to also get used to the type of data.

The intermediate effects database (IEDB) is currently being developed as part of the AOP-KB hub. EU-NETVAL members were asked to consider if they would consider using IEDB and, if so, for which purpose. Members were invited to download IUCLID6 and to try this out.

Overview of the EU-NETVAL survey

A survey was carried out at the beginning of 2016 to assess the training needs within the network and the capacity to offer training. EU-NETVAL members were asked to consult the training report for their permission to decode trainers and trainees, so that laboratories can be brought in contact with each other. All facilities will need to provide their permission to the EU-NETVAL functional mailbox: JRC-ECVAM-NETVAL@ec.europa.eu

For the next meeting participants are invited to proposed topics/methods for training. If the relevant equipment and instrumentation is available, another training session can be organised at the JRC in Ispra.

Introductory session on skin sensitisation methods: h-CLAT, KeratinoSensTM, LuSens and DPRA

EURL ECVAM presented an overview of the skin sensitisation adverse outcome pathway (AOP), demonstrating where the DPRA, KeratinoSens™/LuSens and h-CLAT OECD TG methods fit into this in terms of which key events are addressed. Following the validation of the methods, new insights were available on the ability of the methods to detect pre- and pro- haptens. Approximately 25% of sensitising substances are reported to be pre- or pro- haptens (the majority are pre-haptens). Pre-haptens are generally correctly predicted by *in vitro* methods. Slow oxidisers may not be correctly predicted, just as they would fail to be detected by the *in vivo* methods.

BASF: using the 3 methods as part of a testing battery based on the OECD adverse outcome pathway for skin sensitization

BASF presented how the company uses information from the different *in vitro* skin sensitisation methods for decision making. In the initial phase a QSAR toolbox and a selection of *in vitro* skin methods that represent key events of the skin sensitisation process (i.e. peptide reactivity, keratinocyte activation and dendritic cell activation) were evaluated to identify the combination delivering the best overall accuracy for identification of skin sensitisers. BASF has designed and implemented a strategy for skin sensitisation hazard identification based on two validated OECD TG methods (h-CLAT and DPRA) and the LuSens *in vitro* method. BASF uses a combination of these methods addressing two major steps in the sensitisation process: protein reactivity (DPRA and LuSens) and activation of dendritic cells (h-CLAT). In general, when two out of three tests are positive, the chemical is considered to be a skin sensitiser.

For pre- and pro- haptens the testing strategy must be different, as false negative results can be obtained due to lack of biotransformation. Currently there are no methods available for the fourth skin sensitisation key event 'cell proliferation'. Such methods are complex to develop and perform in the laboratory. However, there is a need for these methods in order to replace the animal test and close cooperation between industry, academia and regulators is required to develop these.

Follow-up discussions on topics raised by partners

- *EU-NETVAL members were asked to suggest topics which they would like to be covered at the next meeting in 2017.*
- *Members were also asked if they would be willing for the film footage of this meeting to be used on our website and shared on CIRCABC.*
- *All members were reminded to notify EURL ECVAM if there are any changes in their organisations/companies.*

Annex I - Agenda

Meeting of EU-NETVAL

1st day: Monday 10th October 2016

Auditorium, Bldg. 58c, Break out group rooms 12a, 12b, Building 101 Rm 2302, 2002

08:15	Bus transfer from hotel to JRC campus
09:00 -11:00	**Welcome and introductory session** - Introduction to the main activities of EURL ECVAM – *Maurice Whelan, EURL ECVAM* - Updates on the current state of the network -*Tracey Holley, EURL ECVAM* - Flash presentations from members (Flash updates – 1 slide 2 minutes) - EURL ECVAM updates: Validation workflow and validation guidance– *Valérie Zuang, EURL ECVAM* AR-CALUX validation project update – *Anne Milcamps and Roman Liska, EURL ECVAM* GIVIMP for the development and implementation of *in vitro* methods for regulatory use in human safety assessment – *Sandra Coecke, EURL ECVAM*
11:00 -11:30	Coffee break
11:30 -12:30	**Presentations from the OECD and the European Commission** Presentation of the OECD Test Guideline programme with focus on *in vitro* methods - *Nathalie Delrue, OECD* Overview of Legislative Anchor - *Suzanna Louhimies, DG Environment*
12:30 -13:30	Lunch
13:30 -15:30	**GIVIMP breakout groups** (*facilitators EURL ECVAM: Sandra Coecke, Gerard Bowe, Lotta Bostroem , Camilla Belmasconi, Ingrid Langezaal, Elisabeth Joossens, Anne Milcamps, Tom Cole, Sander van der Linden EURL ECVAM*) Discussions and breakout groups
15:30 -16:00	Coffee break
16:00 -17:00	**New *in vitro* methods with validation potential** Introduction to survey - *Joao Barroso, EURL ECVAM* Characterization and description of *in vitro* hepatic metabolic clearance methods– *Alfonso Lostia, EURL ECVAM* Thyroid hormone related *in vitro* methods– *Francesca Pistollato, EURL ECVAM*
17:00-17:30	**New reporting approach for *in vitro* methods** OECD Harmonized template 201 used for underpinning AOP building - *Chiara Zorzoli, EURL ECVAM*
17:30 -18:00	**Overview of the EU-NETVAL survey** Training opportunities within the network - *Lotta Bostroem, EURL ECVAM*
18:00	***Session to cover additional topics***
18:30	*Bus transfer to restaurant*

JRC Ispra, 10-11th October 2016

2nd day: Tuesday 11th October 2016

Building 36 Room 03 and EURL ECVAM GLP Test Facility

Trainers - Françoise Brée and Emmanuelle Bazin - Eurosafe; Barbara Birk, Britta Wareing and Carina Oberfrank – BASF; Roger Emter - Givaudan

Facilitators - Emilio Mendoza, Chiara Zorzoli, Ingrid Langezaal, Ann-Charlotte Bostroem, Nikolaos Parissis, Siegfried Morath, Sander van der Linden, Salvador Fortaner, Tom Cole, Anne Milcamps, Sandra Coecke - EURL ECVAM

08:15	Bus transfer from hotel to JRC to **Building 36 Room 03**
09:00 – 09:30	**Introductory session on skin sensitisation methods: h-CLAT, KeratinoSens™, LuSens and DPRA** *Silvia Casati, EURL ECVAM*
	EURL ECVAM GLP Test Facility - Building 101
09:40 – 11:10	**Skin sensitisation knowledge sharing session and practical activities 1**
11:10 – 11:30	Coffee break
11:30 - 13:00	**Skin sensitisation knowledge sharing session and practical activities 2**
13:00 - 14:00	Lunch
14:00 - 14:50	**Skin sensitisation knowledge sharing session – discussion groups (in labs)**
	Building 36 Room 03
15:15 - 16:00	**Plenary session and wrap-up** Presentation from BASF on using the 3 methods as part of a testing battery based on the OECD adverse outcome pathway for skin sensitization – *Barbara Birk, BASF*
16:00 – 16:15	Coffee break
16:15 – 16:30	Concluding remarks and EU-NETVAL suggestions for next year
16:30	End of the Meeting
	Transports to the airport