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JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Validation of Alternative Methods Unit (VAM)
The European Union Reference Laboratory for Alternatives To Animal Testing (EURL ECVAM)

ECVAM SCIENTIFIC ADVISORY COMMITTEE (ESAC)

36th Meeting of the ESAC
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Minutes

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Participants

Present:

1. ESAC Members: David Basketter (ESAC Chair), Nathalie Alépée, Neil Carmichael, Jacques Chretien, Lucio Costa, Rodger Curren, Coenraad Hendriksen, Dagmar Jírova, Erwin Roggen, Vera Rogiers, Andrea Seiler, Kristin Schirmer, Ruud Woutersen.

2. Commission staff: JRC: Joachim Kreysa (HoU VAM), Maurice Whelan (HoU ST), Patric Amcoff (Operational Manager EURL ECVAM), Claudius Griesinger (ESAC/ESTAF Coordination), Juan Riego Sintes (EU Test Method Coordinator), Sharon Munn, Valérie Zuang (Competence Group 1 Leader), Sandra Coecke (Competence Group 2 Leader), Annett Janusch Roi (Competence Group 3 Leader), Maria Pilar Prieto Pereita, Marlies Halder, Silvia Casati, Raffaella Corvi, George Kirmizidis, Susanne Belz, Elise Grignard, Susanne Bremer-Hoffmann ENV: Susanna Louhimies

3. Observers: William Stokes (NICEATM), Hajime Kojima (JaCVAM), Soon Young Han & Young Na Yum (KoCVAM), Nathalie Delrue (OECD Test Guidelines Programme, replacing L. Musset)

Apologies:

1. ESAC Members: Walter Pfaller (Vice Chair), Wallace Hayes

2. Commission staff: JRC: Elke Anklam (Director IHCP), Elisabet Berggren, SANCO: Karin Kilian, Susanne Hoeke, Federica di Gaetano RTD: Arnd Hoeveler, Jürgen Büsing, ENTR: Pieter Dehouck

3. Observers: Laurence Musset (OECD TGP), David Blakey (Health Canada), Jodie Kulpa-Eddy (ICCVAM chair).

1 Agenda point 1: Opening, conflicts of interest

The meeting was opened by a tour de table. The Chair reminded the ESAC members and other participants that all information received/discussed etc. in the context of ESAC's business must be treated with confidentiality, unless otherwise indicated.

The Chair requested whether ESAC members had specific conflicts of interest regarding any of the agenda points of this meeting. Three ESAC members declared interests that had, in part already been stated and reviewed at ESAC 34 and 35.

2 Agenda point 2: ESAC review of the ECVAM follow-up study of the 3T3 NRU assay

DECISION: The draft ESAC opinion prepared by the ESAC Coordinator and ESAC Chair was discussed. Following minor amendments of the document, the ESAC opinion was adopted.

The amendments concerned:

- Executive Summary: Deletion of a half-sentence referring to the high variability of LD50 animal data (as discussed during the ESAC WG work) questioning their suitability as benchmark for validating an in vitro test, in particular when considering positives categorised in several toxicity classes that the animal test data can (due to variability) not resolve. While two ESAC members felt that this sentence should stay in the Executive Summary as referring to a common problem encountered when validating alternative methods in reference to in vivo (animal) data that show high variability (e.g. Draize tests for skin and eye irritation), ESAC finally agreed to delete the half sentence since it was felt that it did not contribute to transmitting the main message of the current opinion which concerned the use of the 3T3 NRU assay for predicting potential negatives.

- Executive Summary: addition of a sentence referring to a weakness of the study design (i.e. a priori exclusion of CMR chemicals from the eligible pool of validation test substances)
- Executive Summary: addition of a sentence clarifying the phrase "chemicals of high toxicity" as substances "in the higher categories of acute oral systemic toxicity".
- Section 2.3: addition of a clarifying sentence concerning the re- calculation of a ROC-based prediction model / thresholds performed by the ESAC WG

Other changes were of minor editorial nature.

It was stressed by ECVAM that, as already communicated at the beginning of the year by the Secretariat and as laid out in the ESAC Rules of Procedure, ESAC Working Groups were charged with drafting (1) the ESAC WG report as well as (2) the ESAC draft opinion. The ESAC Secretariat would in the future not support ESAC by drafting the opinion(s) as had been done in the past (ESAC opinions on carcinogenicity assays and the 3T3 NRU assays). While some ESAC members raised concerns reg. the feasibility of achieving a consistent draft report/opinion without involvement of the ESAC Coordinator/Scientific Secretary, others alerted to the practice in some Commission scientific committees where it is the sole responsibility of the members/rapporteurs to draft all of the documents. There was agreement that approaches were very different, also among Commission scientific committees, and that it was in any case the final responsibility of the Committee to amend and adopt any Committee document as seen fit. The issue was regarded as closed since ECVAM had earlier communicated a standard approach, according to which it was the responsibility of the ESAC WG to draft both the WG report and the ESAC opinion. As a consequence of the discussion it was agreed that, when appointing future ESAC WGs, not only a WG Chair but also an additional WG member should be identified, who should take the lead in drafting the ESAC opinion and ESAC WG report ("rapporteur" tasks).

Based on current review of the 3T3 NRU study, ESAC recommended to ECVAM to avoid excluding a priori specific toxicity classes from validation testing sets (in the present context, CMR substances had been included from the validation testing set). Although ESAC understood that this may be common practice reg. work in some health endpoints and in the interest of the safety of the testing facility personnel, ESAC recommended selecting chemicals primarily in view of the study objective. Study personnel should then be trained appropriately and should take appropriate care when handling the test items, especially in case CMR substances are used as part of a coded validation set of chemicals.

3 Agenda point 3: Status of the ESAC review concerning the Keratinosens study

The current status of the ESAC review of the Keratinosens submission was presented by the ESAC WG Chair and the Secretariat, summarising the issues raised in the context of the correspondence (two letters) between Givaudan (test submitter) and ESAC WG (Dec 2011 – Feb 2012). This correspondence aimed for clarification of issues concerning mainly (1) the statistical analysis of variability on the basis of biological parameters measured and (2) the occurrence of non-qualified runs. While the submission provided plenty of data and information, key information had not been appropriately summarised in the submission document (such as basic performance characterisation on the basis of concordance of predictions (for reliability) and predictive capacity (specificy, sensitivity). This had led to delays in the review. It was discussed that these issues may also stem from the fact that the validation had apparently "evolved" from an initial objective of in-house use, without a clear project plan for the entire information furnished and that the submission consequently constituted a collection of different studies.

Some issues noted were:

- Heterogeneous chemical set employed (lacking full potency range, partly coded, with optimisation continuing during testing, resulting in performance differences between a first and second set);
- Positive control changed at an advanced stage with acceptance criteria (overly strict) not always fulfilled but data still used in statistical analyses anyway.
- Revised statistics report available, with non-qualified tests indicated, but still lacking transparency/explanation of statistical approaches chosen.

The ESAC WG, in its final communication (February 2012) to the submitter has requested a simplified analysis of reliability based on concordance of predictions.

The Secretariat presented the further timelines which consisted of

- a teleconference to discuss the resubmitted analysis of reliability (based on concordance of predictions)
- the WG draft report available for discussion by ESAC and possible feedback to the WG (suggested timeline: 30 May 2012)
- the draft ESAC opinion available to ESAC (suggested timeline: 30 May 2012)

The WG report and draft opinion would be finalised by written procedure.

The ESAC Chair and WG Chair stressed once more that the information submitted by Givaudan was comprehensive, but complex to review. The information, after resubmission of the re-analysed data by Givaudan, appeared now complete in view of finalising the review and concluding on the primary review question, i.e. whether the test method could be considered reproducible. Furthermore it was highlighted that the WG was not mandated to generate an ITS but to identify gaps between study objective / results and conclusions, in particular in view of a later development of an ITS potentially populated by various in vitro test methods and other data sources.

The feasibility of the timelines was discussed. It was agreed that a draft ESAC opinion would be available earliest in June. Taking the summer break into account, the opinion would have to be adopted in July already. This would mean that the process of (1) ESAC feedback to the WG, (2) amendment of the ESAC WG report (if necessary) and (3) finalisation of the ESAC opinion would have to be achieved in less than one month by written procedure. These timelines seemed not achievable. One ESAC member stated that it might be better to foresee more time to reflect on the draft opinion and aim for final adoption at the next ESAC meeting in November 2012 – together with the expected opinion on the DPRA prevalidation study. As the standard project submission form (SPSF) on the Keratinosens had been submitted to OECD already but would, due to the lack of further information (i.e. ESAC opinion/ECVAM recommendation) not be discussed at the upcoming WNT meeting in April 2012, it would seem sufficient to aim for a finalised ESAC opinion and ECVAM recommendation towards the end of 2012 so that these documents could be taken into consideration at the OECD WNT in spring 2013.

DECISION: The Chair summarised that the WG should aim to provide a draft opinion by June and that the subsequent timelines should be handled according to need. The Keratinosens opinion should be latest adopted at the November 2012 meeting of ESAC, together with the DPRA opinion.

4 Agenda point 4: Upcoming review of the Performance Standards of the Cytosensor Microphysiometer test method for eye irritation testing

ECVAM summarised the results of the past ECVAM retrospective validation study of four cytotoxicity/cell-based assays for eye irritation testing, among those the Cytosensor Microphysiometer (CM) method. The validation study and ESAC review (in 2009) had found that the test may be suitable for initiating the top-down approach (i.e. identifying ocular corrosives from the rest of substances) or for initiating the bottom-up approach (i.e. identifying non-classified substances from the rest of chemicals), but was not suitable for resolving mild irritancy. Moreover, the applicability domain of the test methods appeared restricted to water soluble chemicals, particularly surfactants. ECVAM had now developed Performance Standards (PS) for the CM test method for the following reasons:

- ESAC in its 2009 statement had already recommended the development of PS on the basis of the fact that the original equipment of the CM method is not any longer commercially available. Alternative, newer apparatus allowing the measurement of external pH in a flow chamber is however available but would require assessment in view of its capacity to generate comparative results.
- ECVAM has submitted an SPSF to OECD in 2010 and is currently leading the development of a Test Guideline (TG). Some member countries (e.g. Germany) have requested the development of PS as a mandatory requirement for further advancing the development of the TG.
- To allow assessing the suitability and equal performance of new equipment in a consistent manner at least a pre-defined reference chemical set is required. However, it seemed logical to combine efforts towards the definition of a reference chemical set with the additional definition of the essential test method components and target accuracy values. Thus, all three elements of the PS would be defined at one point in time allowing also the assessment of new similar methods (and not only new equipment) in a consistent manner.

The Secretariat briefly summarised the ECVAM request of ESAC advice: ESAC was requested to review the suitability and completeness of the draft ECVAM PS for evaluating the similarity of new methods (and hence whether they qualify for a performance assessment using pre-defined standards) and to judge their performance (reliability, relevance) in reference to the accepted standard laid down in the PS. The Secretariat highlighted that, according to ECVAM, it would be sufficient to charge one or two rapporteurs with the review of the CM PS, instead of setting up an entire ESAC WG.

It followed a discussion on this request. One ESAC member remarked that the new equipment available operated on the same principle as the previous equipment but was based on an improved technology and especially software.

On request of one ESAC member it was clarified that formulations assessed in the retrospective validation study had not been included as part of the performance standards reference chemicals. The reason is that for most of the retrospectively analysed formulations (available literature), the composition was not known.

Following a brief general discussion on the usefulness of PS-based validation studies (questioned by one ESAC member), the Chair invited ESAC to focus and comment on the technical/scientific review of the proposed CM performance standards without digressing on principle questions of (PS-based) validation. In particular the aspect of reliably assessing updated instrumentation should be kept in mind. ESAC was not asked to comment on relative merits of the method versus other methods, nor the relevance of cytotoxicity as a readout/surrogate to predict eye irritation.

The NICEATM observer remarked that it would be useful to involve, in the context of the ICATM collaboration, ICCVAM's ocular toxicity working group (OTWG) in view of getting ICATM views on the draft PS before starting the review process. ECVAM indicated that it would collaborate with the OTWG before finalising the CM PS.

DECISION:

(a) ESAC appointed four ESAC members as rapporteurs charged with preparing the review of the suitability and completeness of the draft CM PS.
(b) The ECVAM Request for ESAC advice including the mandate will be formally amended (if required) and adopted by written procedure, once the final draft CM PS are available.

5 Agenda point 5: ECVAM's approach for advancing and prioritising test methods

ECVAM presented the current advisory structures and operational networks set up in 2009 to 2011 addressing the requirements of Directive 63/2010. It was stressed that EURL-ECVAM's essential role remained the coordination of validation studies – also in collaboration with member states (principle of shared burden) through the so-called NETVAL network of MS laboratories appointed following an official request from the Commission to the MS. Other important elements included the PARERE network (consisting of a MS regulators network and an Interservice Network) and the ESTAF (=ECVAM Stakeholder Forum). Briefly, the intention of ECVAM to work again more on the basis of health endpoints was indicated. Furthermore, the usefulness of the concept of "Adverse Outcome Pathways" (AOPs) for prioritising test methods was currently under discussion. Chemical toxicity in vivo is generally manifested as an integration of specific symptoms, mechanistic stages, physiological susceptibility, etc, where a complement of in vitro methods may be required to cover full health and safety assessments (i.e., for regulatory classification). In general, prioritisation of test submissions aims to improve efficiency and transparency, on three levels: 1) relevance to regulatory policy, 2) relevance as an alternative test, 3) readiness for validation.

It followed a discussion on this presentation.

- ESAC members remarked that criteria for prioritisation had not been outlined and asked whether these had been finalised already. Moreover, one ESAC member suggested that ICATM partner organisations should collaborate in view of developing one consistent set of prioritisation criteria in view of harmonising their procedures and activities. ECVAM clarified that the prioritisation criteria were currently under discussion at ECVAM and had not yet been finalised.
- One ESAC member suggested that ECVAM should organise open calls for test methods addressing a specific health effect or mechanism of action instead of primarily responding to test method submissions. ECVAM explained that this had been discussed already during the last ESTAF meeting (2011) and that

ECVAM was considering such proactive approaches currently for its general approach towards advancing test methods for validation.

- Another ESAC member inquired how the overall approach would speed-up validation as acceleration had been also mentioned as one of the benefits of a renewed approach. ECVAM replied that clear "go/no go" type criteria were required to ensure a swift prioritisation of test methods. It was also made clear that the available resources however were limited.
- The ESAC Chair remarked that the easiest and quickest way to speed up the overall process was to 'devolve' decision-making, i.e. to empower the individuals responsible for advancing particular work with decisions to be made on the basis of clear decision criteria to be developed by management.
- A Commission observer (DG ENV) remarked that the role of PARERE was, according to the provisions of the Directive, very clear and that it would be necessary to ensure that this role remains as its primary core task. However, other additional tasks or work-flows that ECVAM was currently considering for its priority setting are not excluded.

The session continued with an illustration of current prioritisation exercises by work 1) in the area of skin sensitisation and 2) endocrine disruption.

In the area of skin sensitisation, several methods addressing various steps of the OECD-based "Adverse Outcome Pathway" (AOP) for skin sensitisation are currently being evaluated following submission to ECVAM. Others are already under validation by ECVAM (i.e. DPRA, MUSST, h-CLAT). Skin sensitisation provides a well-characterised example of how complementary use of alternative assays would contribute to overall health effect assessment and potency evaluation. Six mechanistic stages have been identified in skin allergic reactions: if one event is critical, an in vitro assay of that particular effect could be decisive in determination of overall adverse outcome pathway (OECD AOP). Various test methods are still in development or have just undergone development and more submissions are expected, in particular as a result of the EU "Sens-it-iv" project. Evaluation focuses currently on mechanistic relevance, assay and data utility, R&D status, 3R impact, etc. Matrix compilation of these criteria will then allow prioritisation for validation and eventual ESAC consultation.

It followed a discussion on the points raised in the presentation:

- ESAC members remarked that it was not sufficiently clear how the AOP for skin sensitisation and possible testing data would help with the categorisation of chemicals.
- ESAC members inquired whether the AOP had been developed based on a wide spectrum of chemical substances or rather on the basis of general physiological / immunological literature.
- One ESAC member inquired whether there were already ideas reg. skin sensitisation ITS. In particular, if one in vitro method would yield a negative result, would this be a final decision point already? ECVAM explained that the strategy still needed development and that these efforts would require well characterised test methods, in particular with regard to their reliability. One ESAC member remarked that reliability was certainly not enough but that test methods also should be characterised in terms of their predictive capacity, their applicability and limitations so as to place them appropriately in a testing strategy.

In the area of endocrine disruption, mainly one mechanism – binding to/transactivation of the estrogen /androgen receptors - is currently addressed by submitted methods (biochemical binding assays and biological transactivation assays using transfected cell lines). Prioritisation of submitted methods is now required using a matrix outlining the advantages/disadvantages of the methods, identifying key events addressed by individual assays.

It followed a discussion on the points raised in the presentation:

- One ESAC member inquired why EPA had discontinued one validation study on an AR (androgen receptor) assay. ECVAM replied that this had been decided due to a shift in priority at EPA.
- One ESAC member remarked that it seemed sufficient to validate one or two methods for ER and AR and develop Performance Standards for subsequent validation of similar methods.
- One member inquired whether any other hormones were also considered, e.g. thyroxin. Another member replied that effects on thyroxin hormone action were considered highly relevant in the field of cosmetics safety evaluation.

- One ESAC member remarked that ED test methods would not safe any animals, but would rather trigger more in vivo experiments. It needed to be ensured that the false positive rate would not be too high as to avoid unnecessary animal experiments and high cost to industry.
- The NICEATM observer suggested considering US EPA's 'Endocrine Disruptor Screening Program' (EDSP) when deciding on priority setting for ED methods.

The session continued with presentations from ECVAM on the status of alternative methods in the areas of repeated-dose toxicity testing, genotoxicity and carcinogenicity testing as well as toxicokinetics testing. These presentations were based on the work of the JRC-coordinated expert group summarising, for DG SANCO, the status of alternatives for cosmetics safety assessment in view of the 2013 deadline (Adler et al., 2010).

6 Agenda point 6: Upcoming review of the Japanese Bhas42 CTA for carcinogenicity testing

The Secretariat introduced the ECVAM request for ESAC advice concerning the Japanese prospective validation study of the Bhas42 Cell Transformation Assay. ECVAM had received, within the ICATM framework, a request from JaCVAM to coordinate/manage the peer review of this study and had agreed to do so after having consulted ESAC. ESAC had agreed to employ the previous ESAC WG CTA for this purpose. The ESAC WG CTA had, in 2010/2011 reviewed the ECVAM-coordinated study on the reliability and transferability of the SHE and Balb/C CTA protocols. The review questions would be set as soon as the adopted Validation Study Report was available. Current indications were July. These were confirmed by JaCVAM.

ECVAM gave a brief overview, on behalf of JaCVAM, on the study. All data had been generated prospectively; no historical data had been used. Importantly, the Bhas42 CTA holds the promise to allow distinguishing initiators and promotors of carcinogenicity. As the validation study comprised two protocols, one conventional six-well design and one using a 96 well design, it may be also amenable for automated testing.

DECISION: Following a brief discussion on the study, ESAC agreed that the two ESAC members in the ESAC WG would focus on one main responsibility, with one member acting as chair and the other one as rapporteur, the latter responsible for drafting the ESAC WG report and draft opinion.

7 Agenda point 7: ECVAM-coordinated Zebrafish Embryotoxicity study

In view of a possible ESAC peer review, ECVAM presented the OECD validation study of the Zebrafish Embryo Toxicity Test (ZFET) and in particular the ECVAM-coordinated part concerning reliability of the assay. The current study was driven by OECD Project 2.7 towards the development of a new TG for a "fish embryotoxicity test"(FET). Background to these activities is OECD TG 203 on acute fish toxicity. The TG's purpose is to allow determination of the LC50 value, i.e. the concentration of a substance dissolved in water which will kill 50% of the fish population exposed. EU statistics from 2008 suggest that 51'000 fish are used annually for LC50 tests. Species used include zebrafish, fathead minnow, Japanese medaka. Substances tested are chemicals, veterinarian pharmaceuticals, plant production products, biocides, food & feed and others.

The ECVAM-coordinated study part focused on the assessment of the transferability, within- and between-laboratory reproducibility of the ZFET, while determination of the predictive capacity was not an objective of the study. The study now required being peer reviewed and it was likely that ECVAM would request ESAC to review it.

In the following discussion, the OECD observer emphasized that according to OECD procedures, peer review was not necessarily a mandatory requirement following completion of a validation study. Often discussions within the relevant expert groups were considered as a substitute for peer review. A formal decision on a) whether or not peer reviews will be conducted and b) which organisation will conduct the peer review would rest with the OECD (i.e. WNT) as this was an OECD study. In any case, an ESAC peer review would be very welcome and expected to support further decision-making at OECD.

ECVAM indicated that, if requested by OECD, would only review the ECVAM-coordinated part of the study.

8 Agenda point 8: ECVAM activity report

The activities of competence groups 1 (validation), 2 (in house assessment and training) and 3 (databases) of the VAM unit were presented. Further a presentation on Lumicell validation study using the automated platform was given by the ST unit.

9 Agenda point 9: ESAC operations and update of ESAC's Rules of Procedure (RoP)

The ESAC Secretariat provided an overview of the current planning of ongoing/upcoming ESAC reviews, i.e. the envisaged timelines for finalising the deliverables and presented some minor changes to ESAC's 'Rules of Procedure' (RoP) that were required following internal JRC audit and which concern possible interests of members, invited experts and observers and the Chair's powers to exclude any participant from discussions, decision taking (if ESAC members are concerned) and written procedure, if deemed necessary.

Furthermore the meeting dates for 2012 and 2013 were again communicated:

- 2012 Autumn meeting:
  Tuesday / Wednesday 6/7 November 2012

- 2013 Spring meeting:
  Tuesday / Wednesday 26/27 March 2013

- 2013 Autumn meeting:
  Tuesday / Wednesday 8/9 October 2013

Finally, the ESAC was reminded that the three years term of office would come to an end in December this year. As membership is renewable, ECVAM would circulate an official letter to ESAC members requesting indications as to whether members would like to be considered for a renewed term. Moreover, ECVAM indicated that it was currently considering to slightly enlarge the ESAC (to 19 members) in view of gathering the necessary expertise.

10 Agenda point 10: Activity reports of ICATM partners and OECD TGP

The ICATM partner organisations (NICEATM/ICCVAM, KoCVAM, JaCVAM) as well as the OECD Test Guidelines Programme (TGP) provided detailed overviews of their work.

11 ACTIONS

Nr.	Agenda item	Action and timeline	Actors
1	AP2 ESAC opinion 3T3 NRU	Action: Distribution of final ESAC opinion on the ECVAM follow-up study concerning the predictive capacity of the 3T3 NRU assay (as adopted during this meeting). Timeline: Within March 2012	• ESAC Secretariat
2	AP4 PS for Cytosensor Microphysiometer	Action: Adoption of the ESAC request for review of the Performance Standards of the Cytosensor Microphysiometer assay by written procedure. Timeline: As soon as final ECVAM draft Performance Standards are available.	• ESAC Secretariat • ESAC
3	AP6 ESAC review of BHAS 42 CTA	Action: Circulation of final ESAC request BHAS42 CTA for comment and adoption Timeline: As soon as possible, pending availability of the final Validation Study Report (VSR), currently expected in July 2012	• ESAC Secretariat • ESAC
4	AP7 Possible ESAC review of ECVAM coordinated part of the OECD study on the Zebrafish Embryotoxicity Test (ZET)	Action: Development of a list of candidate members of a potential ESAC WG for reviewing the zebrafish embryotox test based on a) proposals form ECVAM, b) proposals from ESAC and c) proposals from ICATM. Action: April 2012 and following final decision of OECD WNT concerning the review of the ZFET study.	• ESAC Secretariat
5	AP9 Update of ESAC Rules of Procedure (RoP)	Action: Distribution of final proposal of the update of the ESAC Rules of Procedure. Timeline: Within April 2012	• ESAC Secretariat