ESSENTIAL INFORMATION

In this section, please provide the information required by the Working Group of National Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. What is the existing or expected regulatory need/data requirement that will be met by the proposed outcome of the project? Please provide details below or as an attachment.

The proposed assay will provide data for Level2 assay (in vitro assays providing mechanistic data) in OECD EDTA conceptual framework

or as attachment No.__

2. How will the work contribute to further international harmonisation of hazard and risk assessment? Please provide details below or as an attachment.

Approaches for endocrine disrupters testing and risk assessment will be more harmonized.

or as attachment No.__

3. How will the proposed project address issues and /or endpoints which are of major human health or environmental concerns? Please provide details below or as an attachment.

The proposed assay can be used for the screening of potential endocrine modulating chemicals through ER

or as attachment No.__

4. Will the project have general support from OECD member countries or is the outcome relevant for just one or a few member countries / stakeholders? Provide details of the countries and the rationale for this view below.

☒ Many countries ☐ A few countries ☐ Only for the submitting country

Japan, United States, EU countries

5. If the Test Guideline is not intended for general use, indicate if the Test Guideline would be intended for:

☐ Specific (limited) applications such as pesticide usage, or

☐ for specific classes of chemicals (e.g. surfactants) rather than for chemicals in general.

6. If the expected outcome of this proposal is a Test Guideline or a Guidance Document, provide information on the intended use, applicability and limitations of the test method.

The test method is applicable for use as a Level 2 *in vitro* screening assay as described in the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. Applicability and limitations will be determined taking into account the results of the validation study.

7. Provide supporting information on the validation status (i.e. relevance and reliability) of the method. Principles for validation of test methods for OECD Test Guidelines are described in Guidance Document 34.

Provide justification and rationale for the test, including data.

If there are no or limited data available to support the reliability and relevance of the proposed test, indicate if validation work is included in the project.

If there is no need for validation provide a detailed justification.

The revised draft TG for detecting "estrogenic" activity using HeLa-9903 is under circulation. Since there are urgent needs for detecting anti-estrogenic activity using the validated cell, the pre-validation study for anti-estrogenic activity using HeLa-9903 cell line has been conducted, and highly reproducible results have been obtained using 7 known ER antagonists. The standardized protocol will be formalized in early 2008.

The multi-lab validation study using 10-12 coded chemicals including 3-4 laboratories under the lead of JaCVAM (Japanese Center for the Validation of Alternatives) will be initiated in early 2008. The participation from Japanese and European laboratories is planed.

ADDITIONAL INFORMATION

In this section please provide further information to allow the Working Group of National Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. If the expected outcome of the project proposal is a Test Guideline and is based on existing, regional or international documents such as guidelines, protocols or guidance material, please provide that information here or as an attachment.

ER antagonist effect is one of important mechanisms in endocrine disruption. There is no test guideline for ERTA assay to detect ER antagonist effect of chemicals, although TG of ERTA assay for ER agonist is under consideration in OECD test guideline program. This project will provide the complementary TG for ERTA assay and also will provide additional information to the requirement arisen from the peer review panel of the ERTA agonist assay using HeLa-9903 cell line.

or as attachment No.__

2. If Animal Welfare considerations are addressed in the project proposal, provide details below or as an attachment. Explain if the project is aimed at refining, reducing and/or replacing the use of animals.

If the project is not specifically developed for animal welfare purposes, indicate if the animal welfare considerations have been a component of the project proposal.

Indicate if animal welfare considerations are irrelevant to the project, for example for physico-chemical properties.

Although not aimed at the issue of animal welfare it will certainly be considered

or as attachment No.__

3. Provide information on expected or possible resource savings in member countries as a result of this project.

Harmonized approach for assessment of endocrine disrupters would save time and resources in Member countries.

4. If the expected outcome of the proposed project is a Guidance Document or Detailed Review Paper, will it be directly linked to the development of a particular Test Guideline or a series of Test Guidelines?

☒ Yes, it is the initial step in the development of a new or revision of existing Guidelines.

☐ Yes, additional guidance is needed for the most appropriate selection of the Guidelines on the subject.

☐ No, the guidance is on issues related to testing or the development of Test Guidelines in general.

There are ___ attachments added to this form.

ASSESSMENT OF PROJECT PROPOSAL

(To be completed by all member countries /stakeholders except the submitter)

Country / Organisation:	

Representative: (Preferably NC):	

Taking into account the project information, requested above, does this project meet the needs of the member countries for addition to the workplan of the Test Guidelines Programme

☐ Yes ☐ No ☐ Further information needed

If the response is "No" or "Further information needed", please provide justification:

Remarks as appropriate, including further information needs, if any:

OECD TEST GUIDELINES PROGRAMME

Standard Project Submission Form

If you require further information please contact the OECD Secretariat
Return completed forms to:
env.tgcontact@oecd.org

PROJECT TITLE

Stably transfected Transcriptional Activation (TA) assay for detection of androgenic and anti-androgenic activity of chemicals

SUBMITTED BY (Country / European Commission / Secretariat)

Yumiko Nomura and Ayumi Kodama

DATE OF SUBMISSION TO THE SECRETARIAT

January 2008

DETAILS OF LEAD COUNTRY/CONSORTIUM

Country /Organisation:	Japan
Agency/ministry/Other:	Ministry of Health, Labour and Welfare (MHLW), Japan and Ministry of Economy, Trade and Industry (METI)
Mail Address:	Kasumigaseki 1-2-2, Chiyoda-ku Tokyo, Japan and Kasumigaseki 1-3-1, Chiyoda-ku Tokyo, Japan
Phone/fax:	81-3-3595-2298/81-3-3593-8913 and 81-3-3501-0080/81-3-3580-6347
Email:	nomura-yumiko@mhlw.go.jp and kodama-ayumi@meti.go.jp

PROJECT OUTCOMES

☒ New Test Guideline	☐ Guidance document
☐ Revised Test Guideline	☐ Detailed Review Paper
☐ Deletion of an existing Test Guideline	☐ Other, please specify below

PROPOSED WORK PLAN and RESOURCE NEEDS:

1. Draft workplan for development of the proposal, including any need to establish Ad Hoc Expert Group and mode of meetings (face-to-face, teleconference; electronic discussion group). Indicate key milestones, including first and subsequent drafts of documents and timing of meetings.

The validation study for a method using androgen responsive stable cell line (AR-EcoScreen™) to detect androgenic and anti-androgenic activity of chemicals has been completed and its validation report and its draft guideline will be available in March, 2008. The scientific peer review will be initiated within the 2008 JFY and the report from the scientific peer review will be prepared until early 2009.

2. Will additional information, including generation or collection of data, be required? If yes, please describe the anticipated process and timelines.

Not planned

3. Indicate the estimated overall resource need (time/money) for member country / consortium and Secretariat

- Time: At least, six weeks of peer review process 3-4 weeks for the consultant to prepare and finalize the peer review report from the submission of Validation report and draft test guideline.
- Money: At least, 20,000-EUR would be needed if employ independent consultant for the peer review process.

4. Is this proposal intended to replace an existing Test Guideline or lead to the deletion of an existing Test Guideline?

No

ESSENTIAL INFORMATION

In this section, please provide the information required by the Working Group of National

Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. What is the existing or expected regulatory need/data requirement that will be met by the proposed outcome of the project? Please provide details below or as an attachment.

The proposed assay will provide data for Level2 assay (in vitro assays providing mechanistic data) in OECD EDTA conceptual framework

or as attachment No.__

2. How will the work contribute to further international harmonisation of hazard and risk assessment? Please provide details below or as an attachment.

Approaches for endocrine disrupters testing and risk assessment will be more harmonized.

or as attachment No.__

3. How will the proposed project address issues and /or endpoints which are of major human health or environmental concerns? Please provide details below or as an attachment.

The proposed assay can be used fro the screening of potential endocrine modulating chemicals through AR

or as attachment No.__

4. Will the project have general support from OECD member countries or is the outcome relevant for just one or a few member countries / stakeholders? Provide details of the countries and the rationale for this view below.

☒ Many countries ☐ A few countries ☐ Only for the submitting country

Japan, United States, EU countries

5. If the Test Guideline is not intended for general use, indicate if the Test Guideline would be intended for:

☐ Specific (limited) applications such as pesticide usage, or

☐ for specific classes of chemicals (e.g. surfactants) rather than for chemicals in general.

6. If the expected outcome of this proposal is a Test Guideline or a Guidance Document, provide information on the intended use, applicability and limitations of the test method.

The test method is applicable for use as a Level 2 *in vitro* screening assay as described in the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. Applicability and limitations /////

7. Provide supporting information on the validation status (i.e. relevance and reliability) of the method. Principles for validation of test methods for OECD Test Guidelines are described in Guidance Document 34.

Provide justification and rationale for the test, including data.

If there are no or limited data available to support the reliability and relevance of the proposed test, indicate if validation work is included in the project.

If there is no need for validation provide a detailed justification.

At the present time, there is global concern regarding endocrine disruption effects, particularly mediated by the andorogen receptor (AR) resulting from chemical exposure. Several *in vitro* AR binding and transfected cell line assay methods are currently or imminently being (pre) validated at national, regional and international levels, but are some way away from completion and full assessment of their validation status. Currently, no *in vitro* screening assay for AR activity that can be used for OECD regulatory purposes has been peer reviewed for potential test guideline development, although the need is urgent. Recognizing this urgency, Japan has made an extensive effort to establish and domestically validate a new *in vitro* pre-screening procedure, the Andorogen Receptor (AR) Transcriptional Activation (TA) Test for detecting the andorogenic and anti-andorogenic activities of chemicals for a level 2 screening test in the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. The with-in Japan multi-laboratory validation process of Japanese ER TA Assay was completed as an activity of the Validation Management Group (Non -Animal) (VMG-NA) and the results were presented at the 3rd and 5th VMG-NA held in November 2007.

The assay is based on an andorogen responsive stable cell line, AR EcoScreen cell, which was developed by the Otuka Permaceutical Ltd. in Japan. An initial test protocol of the assay system was developed and optimized in the Otuka Permaceutical. Using the optimized protocol, a pre-validation of the test system was conducted by the same company as an initial assessment exercise in order to identify the reliability, relevance and performance (accuracy) of the assay system. Following this initial assessment of the assay system, CERI led an inter-laboratory validation involving four participating laboratories, all of which used coded chemicals under GLP compliance conditions. The data produced indicated good reproducibility and technical transference between laboratories.

ADDITIONAL INFORMATION

In this section please provide further information to allow the Working Group of National Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. If the expected outcome of the project proposal is a Test Guideline and is based on existing, regional or international documents such as guidelines, protocols or guidance material, please provide that information here or as an attachment.

AR agonist and antagonist effects are one of important mechanisms in endocrine disruption. There is no test guideline for ARTA assay to detect AR agonist and antagonist effect of chemicals, although TG of ERTA assay for ER agonist is under consideration in OECD test guideline program. This project will provide the useful TG to detect AR mediated endocrine

modulating effect.

or as attachment No.__

2. If Animal Welfare considerations are addressed in the project proposal, provide details below or as an attachment. Explain if the project is aimed at refining, reducing and/or replacing the use of animals.

If the project is not specifically developed for animal welfare purposes, indicate if the animal welfare considerations have been a component of the project proposal.

Indicate if animal welfare considerations are irrelevant to the project, for example for physico-chemical properties.

Although not aimed at the issue of animal welfare it will certainly be considered

or as attachment No.__

3. Provide information on expected or possible resource savings in member countries as a result of this project.

Harmonized approach for assessment of endocrine disrupters would save time and resources in Member countries.

4. If the expected outcome of the proposed project is a Guidance Document or Detailed Review Paper, will it be directly linked to the development of a particular Test Guideline or a series of Test Guidelines?

☒ Yes, it is the initial step in the development of a new or revision of existing Guidelines.

☐ Yes, additional guidance is needed for the most appropriate selection of the Guidelines on the subject.

☐ No, the guidance is on issues related to testing or the development of Test Guidelines in general.

There are ___ attachments added to this form.

ASSESSMENT OF PROJECT PROPOSAL

(To be completed by all member countries /stakeholders except the submitter)

Country / Organisation:	

Representative: (Preferably NC):	

Taking into account the project information, requested above, does this project meet the needs of the member countries for addition to the workplan of the Test Guidelines Programme

☐ Yes ☐ No ☐ Further information needed

If the response is "No" or "Further information needed", please provide justification:

Remarks as appropriate, including further information needs, if any:

OECD TEST GUIDELINES PROGRAMME

Standard Project Submission Form

If you require further information please contact the OECD Secretariat
Return completed forms to:
env.tgcontact@oecd.org

PROJECT TITLE

Comet Assay
in Genotoxicity Testing

SUBMITTED BY (Country / European Commission / Secretariat)

Japan(Dr. Yamamoto Japan National Coordinator for the OECD Test Guidelines Program)

DATE OF SUBMISSION TO THE SECRETARIAT

January, **,2008

DETAILS OF LEAD COUNTRY/CONSORTIUM

Country /Organisation:	Japan
Agency/ministry/Other:	Lead Institute: Japanese Centre for the Validation of Alternative Methods (JaCVAM), National Institute of Health Sciences (NIHS) Supporting Ministry: Ministry of Health, Labour and Welfare (MHLW), Japan
Mail Address:	National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-5801, Japan Kasumigaseki 1-2-2, Chiyoda-ku Tokyo, Japan
Phone/fax:	Phone: +81-3-3700-9874 (Kojima); +81-3-3700-9872(Hayashi) Fax: +81-3-3700-9874
Email:	h-kojima@nihs.go.jp (Hajime Kojima, Director, JaCVAM, NIHS) hayashi@nihs.go.jp (Makoto Hayashi, Head, Div. of Genotoxicity, NIHS)

PROJECT OUTCOMES

- ☒ New Test Guideline
- ☐ Revised Test Guideline
- ☐ Deletion of an existing Test Guideline
- ☐ Guidance document
- ☐ Detailed Review Paper
- ☐ Other, please specify below

PROPOSED WORK PLAN and RESOURCE NEEDS:

1. Draft workplan for development of the proposal, including any need to establish Ad Hoc Expert Group and mode of meetings (face-to-face, teleconference; electronic discussion group). Indicate key milestones, including first and subsequent drafts of documents and timing of meetings.

JaCVAM, ECVAM (European Center for the Validation of Alternative Methods) and ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods) are currently managing an international validation study of the comet assay to detect the first stage for genotoxicity in both *in vitro* and *in vivo* test systems. The validation study incorporates essential test method protocol and more than 40 reference chemicals based on recommendation from the members of international validation management team. The Phase I *in vivo* validation study using three chemicals was finalized in winter 2007. If necessary, we can submit these data in spring 2008. The *in vitro* validation study follows from the *in vivo* study. The final validation study is scheduled for completion in autumn 2010. A draft test guideline is scheduled for submission to the OECD secretariat in early 2011.

2. Will additional information, including generation or collection of data, be required? If yes, please describe the anticipated process and timelines.

The ongoing validation study for the comet assay is scheduled for completion in autumn 2010 and a DRP summarizing the results of this study and any other relevant data and information will be completed shortly thereafter. The results of the validation study, accompanied with a draft test guideline, are scheduled for submission to the OECD secretariat in early 2011. The standardized test method protocol currently being used in the validation study is available as Attachments 1 and 2 .

3. Indicate the estimated overall resource need (time/money) for member country / consortium and Secretariat

Funding for the validation study, the costs associated with the meeting and travel expenses associated with the international validation management meeting is being provided by JaCVAM. JaCVAM resources will be used for study management and development of the BRD.

4. Is this proposal intended to replace an existing Test Guideline or lead to the deletion of an existing Test Guideline?

This project is for the development of a new Test Guideline. We would like to evaluate the use of the *in vitro* and *in vivo* comet assay (multiple tissues) for the assessment of DNA damage by chemicals in order to investigate the correlation with carcinogenicity data at the target organs. The *in vivo* comet assay may be validated as an alternative follow-up assay to the more commonly used *in vivo* liver UDS assay.

ESSENTIAL INFORMATION

In this section, please provide the information required by the Working Group of National Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. What is the existing or expected regulatory need/data requirement that will be met by the proposed outcome of the project? Please provide details below or as an attachment.

The proposed Test Guideline will be used to meet the regulatory needs to evaluate genotoxicity of chemicals.

or as attachment No.__

2. How will the work contribute to further international harmonisation of hazard and risk assessment? Please provide details below or as an attachment.

It is proposed that the comet assay be adopted as a second *in vivo* genotoxicity test in the revised ICH-S2 draft guidance. A flow chart for the *in vivo* comet assay for genotoxicity.

or as attachment No.__

3. How will the proposed project address issues and /or endpoints which are of major human health or environmental concerns? Please provide details below or as an attachment.

The *in vitro* assay will provide a measure of the ability of a test chemical to directly interact with DNA to cause the first step of genotoxicity. The *in vivo* assay can be applied to any tissues in the body regardless of the mitogenic activity, which is required for the cytogenetic assays. This advantage of the method is essential for evaluating the mechanism of carcinogenicity at the target site.

or as attachment No.__

4. Will the project have general support from OECD member countries or is the outcome relevant for just one or a few member countries / stakeholders? Provide details of the countries and the rationale for this view below.

☒ Many countries ☐ A few countries ☐ Only for the submitting country

The method has already been used in many countries for chemical safety assessment. It is, however, not authorized for regulatory use, because the standardized protocol has not been established. Our international validation trial will establish a standard protocol that has been anticipated by many member countries.

5. If the Test Guideline is not intended for general use, indicate if the Test Guideline would be intended for:

☐ Specific (limited) applications such as pesticide usage, or

☐ for specific classes of chemicals (e.g. surfactants) rather than for chemicals in general.

6. If the expected outcome of this proposal is a Test Guideline or a Guidance Document, provide information on the intended use, applicability and limitations of the test method.

The test method is applicable to, as a rule, any kinds of chemical requiring an *in vivo* screen for genotoxicity as well as *in vivo* assays. In the *in vivo* assay, generally it is applicable to any species and any tissues regardless of its karyotype and mitotic activity. The method is rather simple and does not require any sophisticated equipment but needs electrophoresis equipment and preferably an image analyzer with image capture unit.

7. Provide supporting information on the validation status (i.e. relevance and reliability) of the method. Principles for validation of test methods for OECD Test Guidelines are described in Guidance Document 34.

Provide justification and rationale for the test, including data.

If there are no or limited data available to support the reliability and relevance of the proposed test, indicate if validation work is included in the project.

If there is no need for validation provide a detailed justification.

JaCVAM, ECVAM, and ICCVAM are currently managing an international validation study of the comet assay to detect DNA damage *in vitro* and *in vivo* and the validation study is conducted in accordance with the OECD Guidance Document 34. The validation study is scheduled for completion in autumn 2010. At the moment, we are ready to provide the data of phase 1 study at any time on request.

ADDITIONAL INFORMATION

In this section please provide further information to allow the Working Group of National Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. If the expected outcome of the project proposal is a Test Guideline and is based on existing, regional or international documents such as guidelines, protocols or guidance material, please provide that information here or as an attachment.

JaCVAM, ECVAM and ICCVAM are currently managing an international validation study of the *in vivo* and *in vitro* comet assays. The standardized comet assay protocol for the validation study proposed by the validation management team is attached as attachment No. 1 and No. 2, for *in vivo* and *in vitro* methods, respectively.

or as attachment No. 1 & 2

2. If Animal Welfare considerations are addressed in the project proposal, provide details below or as an attachment. Explain if the project is aimed at refining, reducing and/or replacing the use of animals.

If the project is not specifically developed for animal welfare purposes, indicate if the animal welfare considerations have been a component of the project proposal.

Indicate if animal welfare considerations are irrelevant to the project, for example for physico-chemical properties.

The *in vitro* comet assay, as an alternative to the *in vivo* comet assay, can provide information of the direct interaction with DNA as a characteristic of the chemical tested, which is the first step of genotoxicity. The positive result of the *in vitro* comet assay might give important information to evaluate the relevancy of any positive outcomes from the other *in vitro*/*in vivo* genotoxicity assays.This reduces the unnecessary follow-up *in vivo* assays for the chemical safety evaluation. For the *in vivo* assay, it is expected to obtain the essential information of whether carcinogens are genotoxic or non-genotoxic at the target site. The standard test protocol aims to use the minimum number of animals to obtain results with reasonable statistical power.

or as attachment No.__

3. Provide information on expected or possible resource savings in member countries as a result of this project.

The proposed test method enables efficient *in vitro* genotoxicity screening of large numbers of chemicals. In addition, results from this method can be used in weight-of-evidence approach to reduce the number of *in vivo* tests required, especially with the mechanistic analysis for carcinogens, which would also result in resource savings.

4. If the expected outcome of the proposed project is a Guidance Document or Detailed Review Paper, will it be directly linked to the development of a particular Test Guideline or a series of Test Guidelines?

☐ Yes, it is the initial step in the development of a new or revision of existing Guidelines.

☐ Yes, additional guidance is needed for the most appropriate selection of the Guidelines on the subject.

☐ No, the guidance is on issues related to testing or the development of Test Guidelines in general.

There are __2__ attachments added to this form.

ASSESSMENT OF PROJECT PROPOSAL

(To be completed by all member countries /stakeholders except the submitter)

Country / Organisation:	

Representative: (Preferably NC):	

Taking into account the project information, requested above, does this project meet the needs of the member countries for addition to the workplan of the Test Guidelines Programme

☐ Yes ☐ No ☐ Further information needed

If the response is "No" or "Further information needed", please provide justification:

Remarks as appropriate, including further information needs, if any:

(REV 1) Paris 13 November 2008
Patric.Amcoff@oecd.org

DRAFT REPORT OF THE OECD EXPERT CONSULTATION MEETING FOR THE REVISION OF THE DRAFT *IN VITRO* SKIN IRRITATION TEST GUIDELINE

20-21 October, 2008, Federal Institute for Risk Assessment (BfR), Berlin-Marienfelde, Germany

INTRODUCTION

1. The Secretariat opened the meeting and OECD acknowledged the Federal Institute for Risk Assessment (BfR) for once again hosting an expert meeting and introduced Professor Horst Spielmann, who gave a presentation on the history and activities of the BfR. Valérie Zuang of the European Commission (EC has the project lead) introduced the topic and gave a presentation on the background to the ECVAM Skin Irritation Validation Study (SIVS).

2. The Secretariat welcomed participants and described OECD procedures and the work of the Test Guidelines Programme and emphasized that participants ideally are present as experts, independent of any national position. It was also emphasised that due to the animal testing and marketing bans coming into force in EU in March 2009 as imposed by the 7th Amendment to the Cosmetics Directive, there was a real time constraint to be able to meet this deadline for the EC. The Secretariat explained that there probably have to be two parallel processes, one for the EU and one for the OECD, for the Test Guideline developmental process. The Secretariat introduced Horst Spielmann as the acting co-chair together with the Secretariat, which was approved by the meeting.

3. The draft Agenda was slightly revised to also accommodate presentations by Elke Genschow (BfR) and Hajime Kojima (MHLW JaCVAM), see Annex 1 for an agenda.

4. Experts introduced themselves to the meeting (for a list of participants see Annex 2).

5. Karen Hamernick (US EPA) explained that the US had considerable problems with adequately reviewing the proposed draft Test Guideline and the presented analyses and data, including the validation studies due to the limited time given prior to the meeting. She further explained that the US will not endorse or make any concluding decisions on the TG during the meeting and a primary comment was that the TG does not meet US regulatory needs for a number of applications. She will listen and take the information from the meeting back to relevant stakeholders in the US. The Secretariat concurred that the time to review the documents related to the validation of the modified EpiDerm™ assay and the similar SkinEthic RHE™ assay was extremely short, since these validation documents were not available until a few days before the meeting due to the fact that they still were in the ESAC peer review process. It should also be emphasised that the recalculation results of the classifications of the chemicals in the SIVS validation study (when the cut-off value was changed from 2.0 to 2.3), as were presented by Elke Genshow at the meeting where not available for review prior to the meeting. The strategy from the Secretariat is that usually the supporting documentation should be available to experts for their review 4-6 weeks before a meeting, but at this special occasion that deadline was not possible to meet.

PRESENTATIONS AND DISCUSSIONS ON THE PERFORMANCE OF THE THREE TESTS

6. Valérie Zuang noted prior to her presentation that the draft Test Guideline submitted to the OECD, together with the SPSF and accompanying documents in January 2008, was based on the results of the

ECVAM Skin Irritation Validation Study. She gave a presentation on the *"Outcome of the ECVAM Validation Study on In Vitro Tests for Acute Skin Irritation."* The outcome of the study was reviewed by ESAC (ECVAM Scientific Advisory Committee) that issued a statement of scientific validity in April 2007 saying that: *...the EPISKIN™ method showed evidence of being a reliable and relevant stand-alone test for predicting rabbit skin irritation, when the endpoint is evaluated by MTT reduction, and for being used as a replacement for the Draize Skin Irritation Test (OECD TG 404 & Method B.4 of Annex V to Directive 67/548/EEC) for the purposes of distinguishing between R38 skin irritating and no-label (non-skin irritating) test substances. At the present time the IL-1α endpoint was regarded as a useful adjunct to the MTT assay, as it has the potential to increase the sensitivity of the test, without reducing its specificity. This endpoint could be used to confirm negatives obtained with the MTT endpoint. At this time, due to its high specificity, the EpiDerm model reliably identifies skin irritants, but negative results may require further testing (e.g. according to the tiered strategy, as described in the OECD TG 404). Improvement of the EpiDerm protocol should be made to increase the level of sensitivity...."*

7. She also explained that the reason for inclusion of the interleukin 1 alpha (IL-1α) release endpoint in the SIVS was to check R38-classified borderline chemicals (which were the ones that were mostly mis-classified). The prediction model for IL-1α in her presentation had been developed post-hoc. Inclusion of the IL-1α endpoint in the EpiSkin™ protocol considerably increased the sensitivity of the test without significantly decreasing its specificity. For EpiDerm™ no improvement to the outcome was obtained. It is important to note however, that the IL-1α endpoint did not show adequate between-laboratory reproducibility. The meeting reconfirmed the previous decision during the telephone conference call 2 weeks before the meeting, not to include the IL-1α endpoint in the draft Test Guideline, because it is not regarded sufficiently validated.

8. Manfred Liebsch (BfR) presented the SIVS follow-up study in four laboratories with an updated EpiDerm™ Skin Irritataion Test (SIT) protocol and the supporting documents. He especially mentioned Meeting document # 5.1, "Test submission template", as a core document. While basic elements of the protocol (dose and post-incubation period) were kept unchanged, according to the improved barrier function of the EpiDerm™ model, the chemical exposure time had to be increased to 60 minutes to achieve the required sensitivity. Of the 60 minutes, for technical reasons, 25 minutes were performed at room temperature, and 35 minutes were performed at 37°C / 5% CO_2 in the incubator. While the latter change (37° C incubation) did not change the predictive performance, it reduced the data variability. Fiftynine chemicals were tested with this protocol in phase 1 at MatTek, and the 20 reference chemicals were then tested blind in three experienced and one naïve laboratory. In summary, based on the major call across laboratories, the two referenece chemicals classified false positive in the validated reference test (EPISKIN™) were confirmed false positive in EpiDerm™, while of the three chemicals classified false negative in the validated reference test, two were confirmed false negative, and one (#12, terpenylacetate) was correctly identified in all four laboratories as irritant chemical. Thus, a sensitivity and specificity of both 80% was achieved and the reqirement to be equal or better than the validated reference test) was met. Regarding the IL-1α endpoint, it was tested with the 20 chemicals for the optimised EpiDerm™, and it gave no contribution to the performance of the test method.

9. It should be noted that both the validation document packages (CORRELATE Submission Package) for the modified EpiDerm™ and SkinEthic RHE™ were kindly made available to the meeting by ECVAM. This is usually not possible prior to the finalisation of the ESAC peer review process and the issue of an ESAC statement. However, since a decision by the peer review panel had already been taken, there was no real constraints not to make the documents available to the meeting.

10. Manfred Liebsch pointed out that EpiDerm™, EpiSkin™ and SkinEthic RHE™ all used basically the same protocol during the validation, with only minor deviations. The purpose of the EpiDerm™ follow-up validation study was to increase the test sensitivity by an increase of the exposure time from 15

minutes to 60 minutes..Likewise, an exposure period of 42 minutes was optimal for the SkinEthic model to achieve results comparable with the validated reference EPISKIN. Horst Spielmann concluded that only the exposure volumes and exposure times needed to be optimised for each individual epidermis model according to the barrier function, while the basic elements, a of the method, a post-incubation periopd of 42 hrs and the prediction model were identical for all reconstructed epidermis models.

11. Since several chemicals are irritant on rabbit only (Meeting document #6 and presentation slide 8), a discussion followed on the use of human skin patch data and the usability of this data. Horst Spielmann mentioned that the rabbit data is very conservative and over-predict many chemicals compared to human patch data. However, since the predictive 4hr human patch test failed to be adopted as an OECD Test Guideline in 1996 for legislative reasons even the scientific discussions of the human patch data is not easy.

12. A discussion on the usability of the proposed Test Guideline and whether it could be applied by US agencies was initiated by Karen Hamernick. In the US, corrosives and irritants are always tested together in the rabbit test and the skin corrosion Test Guidelines 430, 431 and 435 are only used for positive screening by US agencies. Manfred Liebsch explained the tiered testing strategy outlined in UN GHS, which is also attached to TG 403/404, where you may go from validated in vitro tests to final testing in the rabbit, if necessary. According to Karen Hamernick, the UN GHS system has not been implemented in the US. Karen further wondered if the model could be used for both irritation and corrosion testing. Manfred Liebsch explained that that, although the protocols of the skin corrosion test (SCT) and the skin irritation test (SIT) are both employing MTT as endpoint, they are entirely different: while the MTT assay performed immediately after short term chemical exposure is specific for corrosive effects, only substances predicted "non corrosive" in the SCT should be tested in the SIT to discriminate irriants from non-irritants. Of course all corrosives are also positive in the SIT, and a discrimination of corrosives and irritants would not be possible if only the SIT is performed.

13. Karen Hamernick also raised a concern regarding false negatives obtained with the *in vitro* corrosivity assays during the validation studies. Joao Barroso replied that most of these were either direct MTT reducers, or classified corrosives according to pH class under the testing strategy appended to OECD TG 404.

14. Nathalie Alepée (L'Oréal) presented the SkinEthic RHE™ validation study and explained that only exposure time (42 minutes) is different from the other two tests. In phase 1, 20 chemicals from the SIVS were tested in three laboratories with very good reproducibility. In phase 2, the same 20 chemicals (now coded) were tested in the same three laboratories with equally good reproducibility and a very good predictive performance: 90% sensitivity and 80 specificity were obtained, both in single laboratories and across all laboratories. The IL-1α endpoint did not contribute to the performance. The optimisation to 42 minutes exposure time was done with another set of chemicals before the 20 were tested.

15. Elke Genshow (BfR) presented for the studies preceeding the SIVS and those following the SIVS a re-calculation of the classification data obtained according to the change of the EU GHS cut-off value from 2.0 to 2.3 for GHS category 2. The EU will adopt only category 2 of the GHS and drop the category for weak irritants (category 3). Expectedly, the general trend of the data for all skin models was that sensitivity goes up, specificity goes down and the accuracy goes down slightly. Since the document provided in advance to the ECM suffered a bit from the fact that also studies preceeding the SIVS were called "optimisation studies", the document will be restructured and calculations from ECVAM added, and provided as a joint BfR-ECVAM document for the envisaged expert consultation in the USA (see para 15).

16. Following a new discussion on the usability of the Test Guideline for US agencies, the Secretariat suggested that in order to have an appropriate review of the existing data and the regulatory needs of OECD member countries, an Expert meeting could be held in US sometime early 2009. That would give member countries time to properly review the proposal and discuss the data and the draft Test Guideline in