ANNEX 2

ASSESSMENT OF THE PERFOMANCE CHARACTERISTICS OF PROPOSED *IN VITRO* RECONSTRUCTED HUMAN EPIDERMIS (hRE) MODELS FOR SKIN IRRITATION

INTRODUCTION

1. Test methods proposed for use under this Test Guideline should be evaluated to determine their reliability and accuracy using substances representing the full range of Draize irritancy scores. When evaluated using the 20 recommended reference chemicals (Table 1), the proposed test methods should have reliability and accuracy values which are comparable to that of the validated reference method 1 (Table 2)(14). The accuracy and reliability standards that should be achieved are provided in paragraphs 9 and 10. Non-classified and classified (UN GHS category 2) substances, representing relevant chemical classes are included, so that the reliability and predictive values (sensitivity, specificity, false negative rates, and false positive rates and accuracy) of the proposed test method can be compared to that of the validated reference method 1. The reliability of the test method, as well as its ability to correctly identify UN GHS category 2 irritant substances, should be determined prior to its use for testing new substances.

PERFORMANCE STANDARDS

2. The Performance Standards comprise the following three elements I) Essential Test Method Components, II) Reference Chemicals and III) Defined Accuracy and Reliability Values (7). These Performance Standards are based on the Performance Standards defined after the completion of the ECVAM skin irritation validation study (6).

I) Essential Test Method Components

3. The essential test method components are described in detail in paragraphs 14 to 19 of the Test Guideline and testing should be performed according to the following:

The general model conditions (paragraph 14)

The functional model conditions includes;

- viability (paragraph 15);
- barrier function (paragraph 16);
- morphology (paragraph 17);
- reproducibility (paragraph 18); and,
- quality controls of the model (paragraph 19).

II) Reference Chemicals

4. Reference chemicals are used to determine if the reliability and accuracy of a proposed novel *in vitro* hRE test method, proven to be structurally and functionally sufficiently similar to the validated reference methods, or representing a minor modification of a validated reference method, shows comparable performance to that of the validated reference method 1 (14). The 20 reference chemicals listed

in Table 1 include chemicals representing different chemical classes of interest, as well as substances in UN GHS category 2. The chemicals included in this list comprise 10 UN GHS category 2 chemicals, 3 UN GHS optional category 3 chemicals and 7 non-categorised chemicals. Under this Test Guideline, the optional category 3 is considered as no category. These reference chemicals represent the minimum number of chemicals that should be used to evaluate the accuracy and reliability of a proposed hRE test method for skin irritation. In situations where a listed chemical is unavailable, other chemicals for which adequate *in vivo* reference data are available could be used. If desired, additional chemicals representing other chemical classes and for which adequate *in vivo* reference data are available may be added to the minimum list of reference chemicals to further evaluate the accuracy of the proposed test method.

Table 1. Reference Chemicals for determination of Accuracy and Reliability Values for RECONSTRUCTED HUMAN EPIDERMIS (hRE) Skin Irritation Models*

Chemical*	CAS Number	EINECS No	Physical state	*In vivo* score	GHS *in vitro* cat.	GHS *in vivo* cat.
1-bromo-4-chlorobutane	6940-78-9	230-089-3	L	0	Cat. 2	No Cat.
diethyl phthalate	84-66-2	201-550-6	L	0	No Cat.	No Cat.
naphthalene acetic acid	86-87-3	201-705-8	S	0	No Cat.	No Cat.
allyl phenoxy-acetate	7493-74-5	231-335-2	L	0.3	No Cat.	No Cat.
isopropanol	67-63-0	200-661-7	L	0.3	No Cat.	No Cat.
4-methyl-thio-benzaldehyde	3446-89-7	222-365-7	L	1	Cat. 2	No Cat.
methyl stearate	112-61-8	203-990-4	S	1	No Cat.	No Cat.
heptyl butyrate	5870-93-9	227-526-5	L	1.7	No Cat.	Optional Cat. 3
hexyl salicylate	6259-76-3	228-408-6	L	2	No Cat.	Optional Cat. 3
tri-isobutyl phosphate	126-71-6	204-798-3	L	2	Cat. 2	Optional Cat. 3
1-decanol	112-30-1	203-956-9	L	2.3	Cat. 2	Cat. 2
cyclamen aldehyde	103-95-7	203-161-7	L	2.3	Cat. 2	Cat. 2
1-bromohexane	111-25-1	203-850-2	L	2.7	Cat. 2	Cat. 2
2-chloromethyl-3,5-dimethyl-4-methoxypyridine hydrochloride	86604-75-3	434-680-9	S	2.7	Cat. 2	Cat. 2
a-terpineol	98-55-5	202-680-6	L	2.7	Cat. 2	Cat. 2
di-n-propyl disulphide	629-19-6	211-079-8	L	3	No Cat.	Cat. 2
butyl methacrylate	97-88-1	202-615-1	L	3	Cat. 2	Cat. 2

benzenethiol, 5-(1,1-dimethylethyl)-2-methyl	7340-90-1	438-520-9	L	3.3	Cat. 2	Cat. 2
1-methyl-3-phenyl-1-piperazine	5271-27-2	431-180-2	S	3.3	Cat. 2	Cat. 2
heptanal	111-71-7	203-898-4	L	4	Cat. 2	Cat. 2

* The chemicals listed in Table 2 provide a representative distribution of the 58 chemicals used in the ECVAM international skin irritation validation study (16). A complete list of test substances and the criteria for their selection are available (5). Their selection is based on the following criteria:

1. the chemicals are commercially available
2. they are representative of the full range of Draize irritancy scores (from non-irritant to strong irritant)
3. they have a well-defined chemical structure
4. they are representative of the validated method's reproducibility and predictive capacity as determined in the ECVAM validation study
5. they are representative of the chemical functionality used in the validation process
6. they are not associated with an extremely toxic profile (e.g. carcinogenic or toxic to the reproductive system) and they are not associated with prohibitive disposal costs.

III) Defined Accuracy and Reliability Values

5. The predictive values (sensitivity, specificity, false negative rate, false positive rate and accuracy) of the proposed test method should be at least comparable to that of the validated reference method 1 (Table 2), i.e., sensitivity should be equal or higher (≥) than 80%, specificity should be equal or higher (≥) than 70%, and accuracy should be equal or higher (≥) than 75%. The calculation of the predictive values should be done using all classifications obtained for the 20 chemicals in the different participating laboratories. The classification for each chemical in each laboratory should be obtained by using the mean value of viability over the different runs performed (minimum three valid runs).

Table 2. Predictive values of the Validated Reference Method 1[1]

Test method	No. of Chemicals	Sensitivity	Specificity	False Negative Rate	False Positive rate	Accuracy
Validated Reference Method 1[2]	58	87.2%	71.1%	12.8%	29.9%	74.7%
Validated Reference Method 1[2]	20	90%	73.3%	10%	26.7%	81.7%

[1] Table 3 provides the predictive values of the validated reference method 1, with regard to its ability to correctly identify irritant chemicals (UN GHS category 2) and non-classified chemicals (no category including optional category 3) for the 58 and 20 Reference Chemicals in Table 1, respectively.
[2] EpiSkin™

6. The reliability of the proposed test method should be comparable to that of the validated reference methods.

Within-laboratory reproducibility

7. An assessment of within-laboratory variability should show a concordance of classifications

(category 2/no category) obtained in different, independent test runs of the 20 Reference Chemicals within one single laboratory equal or higher (≥) than 90%.

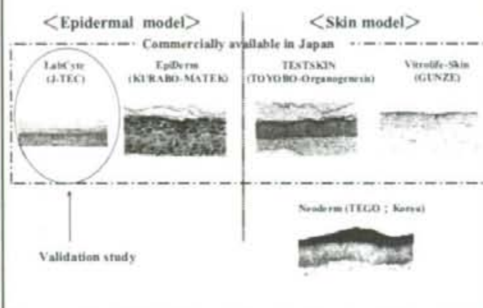
Between-laboratory reproducibility

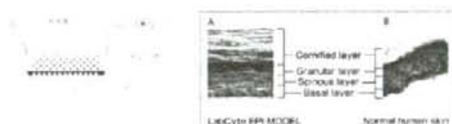
8. An assessment of between-laboratory reproducibility is not essential if the proposed test method is to be used in one laboratory only. For methods to be transferred between laboratories, the concordance of classifications (category 2 / no category) obtained in different, independent test runs of the 20 Reference Chemicals between preferentially a minimum of three laboratories should be equal or higher (≥) than 80%.

New Japanese Validation study in 2008

Hajime Kojima,
JaCVAM (Japanese Center for the Validation of Alternative Methods)

Three-dimensional human skin models in Japan





The LabCyte EPI-MODEL is produced by culturing human epidermal cells on a culture plate. After human epidermal cells have been cultured and proliferated, exposing their surface to the air causes it to keratinize*, creating a cultured epidermis model similar to the human epidermis (Figures A and B).

Substances using the LabCyte validation study

No.	Chemicals	CAS number	E U Level	E U In vivo score
1	1-bromo-4-chlorobutane	6940-78-9	no	0
2	diethyl phthalate	84-66-2	no	0
3	di-propylene glycol	25265-71-8	no	0
4	naphthalen acetic acid	86-87-3	no	0
5	allyl phenoxy-acetate	7493-74-5	no	0.3
6	isopropanol	67-63-0	no	0.3
7	4-methyl-thio-benzaldehyde	3446-89-7	no	1
8	methyl stearate	112-61-8	no	1
9	allyl heptanoate	142-19-8	no	1.7
10	heptyl butyrate	5870-93-9	no	1.7
11	hexyl salicylate	6259-76-3	R38	2
12	terpinyl acetate	80-26-2	R38	2
13				
	5(W/V %) SLS	151-21-3		
14	1-decanol	112-30-1	R38	2.3
15	cyclamen aldehyde	103-95-7	R38	2.3
16	1-bromohexane	111-25-1	R38	2.7
17	α-terpineol	98-55-5	R38	2.7
18	di-n-propyl disulphide	629-19-6	R38	3
19	butyl methacrylate	97-88-1	R38	3
20	heptanal	111-71-7	R38	4
NC	Distilled water			
PC	5(W/V %) SLS	151-21-3		

【Reliability in Leading Laboratory】
MTT

		In vivo classification Irritant	Not Irritant	Total
In vitro prediction	Irritant	6	2	8
	Not irritant	3	8	11
	Total	9	10	19

Sensitivity (%)	66.7
Specificity (%)	80.0
Positive predictive value (%)	75.0
Negative predictive value (%)	72.7
Accuracy (%)	**73.7**

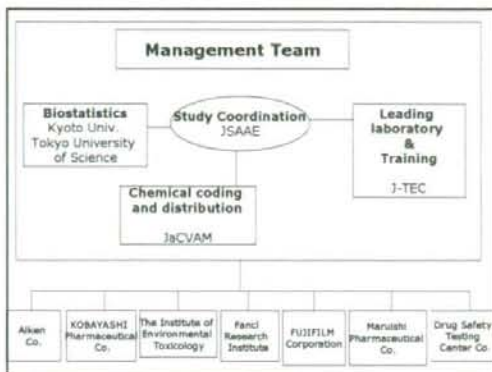
【Reliability in Leading Laboratory】
MTT and IL-1α

		In vivo classification Irritant	Not Irritant	Total
In vitro prediction	Irritant	7	2	9
	Not irritant	2	8	10
	Total	9	10	19

Sensitivity (%)	77.8
Specificity (%)	80.0
Positive predictive value (%)	77.8
Negative predictive value (%)	80.0
Accuracy (%)	**78.9**

Validated 2008: Skin irritation

Type	Me-too assay using Japanese 3-dimensional Epidermal model (LabCyte)
Developer	J-TEC
Coordinator	JSAAE (Japanese Society for Alternative to Animal Experiments)
Support	JaCVAM
Labs.	Seven (only Japanese companies)
SOP	EPISKIN performance standard 15 min. exposure + 42 hr post incubation
Chemicals	19 (refer to ECVAM performance standard)
Deadline	1st step July in 2008 2nd step December in 2008



【Difference betweeen EpiSkin SOP and LabCyte SOP】

1. Volume of culture medium

	LabCyte EPI-MODEL24 SOP	EpiSkin SOP
Pre-culture	0.5mL	2mL
Post-culture	1mL	2mL
MTT assay	0.5mL	2mL

Performance Standard: Not described

2. Volume of test substances

Aspect	LabCyte EPI-MODEL24 SOP	EpiSkin SOP
Liquid	25μL (75μL/cm²)	10μL (26μL/cm²)
Solid	25mg+25μL D.W. (75μ /cm²)	10mg+10μL D.W (26μ /cm²)

Performance Standard : ≧ 25μg/cm²

3. Positive criteria of IL-1α measurements

LabCyte EPI-MODEL24 SOP	EpiSkin SOP	Performance Standard (EpiSkin)
IL-1α release ≧ 130pg/model (IL-1α release ≧ 130pg/mL)	IL-1α release ≧ 100pg/model (IL-1α release ≧ 50pg/mL)	IL-1α release ≧ 120pg/model (IL-1α release ≧ 60pg/mL)

Data of Step 1

		1	2	3		
Chem.	Lab.	Viability	Viability	Viability	Mean	SD
PC	a	6.30	27.30	15.87	16.52	10.63
	b	3.94	2.51	3.07	3.01	0.29
	c	5.45	4.21	2.40	4.56	1
	d	11.74	7.22	14.06	11.02	3.40
	e	31.6	5.75	38.41	25.56	13.05
	f	2.7	2.85	2.52	2.57	0.11
	g	4.40	1.17	7.62	4.73	2.25
PS1 Ethanol	a	57.57	26.12	48.61	43.48	17.03
	b	41.06	50.00	88.58	59.21	23.95
	c	86.12	34.13	57.31	58.53	15.4
	d	98.17	40.52	23.03	47.31	16.12
	e	54.15	72.06	80.55	62.27	9.07
	f		64.16	47.98	56.07	11.44
	g	4.66	3.23	6.87	5.53	1.03
PS2 Glycerol	a	103.63	104.17	98.45	102.09	3.14
	b	83.3	100.56	81.37	88.88	10.33
	c	101.29	99.41	104.94	101.83	2.76
	d	103.3	101.25	88.72	99.13	1.24
	e	101.75	98.06	99.04	99.62	1.91
	f		97.72	96	96.87	0.81
	g	94	98.16	102.8	98.33	4.42
PS3 [illegible]	a	106.13	90.72	91.76	93.27	9.75
	b	93.96	102.91	103.96	100.61	5.76
	c	103.88	108.11	111.2	107.83	3.36
	d	102.28	98.13	94.56	98.32	3.88
	e	102.11	104.29	97.36	102.95	3.03
	f		101.34	102.07	101.7	0.52
	g	97.2	101.04	100.52	99.59	4.76

Future Presentation

November 13-14, 2008
21st Annual meeting on Japanese Society for Alternative ton Animal Experiments, Saitama, Japan

March, 2009
SOT, Baltimore, USA

June, 2009
JST, Iwate, Japan

September, 2009
7th World Congress, Rome, Italy

Thank you for your attention!

Paris, 12 January 2009
Patric.Amcoff@oecd.org

REPORT OF THE 6TH MEETING OF THE VALIDATION MANAGEMENT GROUP FOR NON-ANIMAL TESTING (VMG-NA6)

19-21 November 2008, OECD, Paris

INTRODUCTION

1. The 6th Meeting of the Validation Management Group for Non-Animal Tests (VMG-NA) was held in Paris, France on 19th-21th November 2008 in the new Conference Building at the OECD. The main objective of the VMG-NA is to identify or propose validated or promising non-animal assays for endocrine active chemicals testing, and develop and validate tools necessary for the Level 2 (*In vitro* assays providing mechanistic data) of the Conceptual Framework of the Endocrine Disruption Testing and Assessment Task Force of the Test Guidelines Programme (EDTA).

2. The Secretariat opened the Meeting and welcomed participants of the VMG-NA6. The OECD procedures were explained and Dr. Steve Bradbury (US EPA) and Dr. Daniel Dietrich (Konstanz University, Germany) were introduced as the co-chairs of the meeting.

ADOPTION OF THE DRAFT AGENDA

3. The Secretariat introduced the agenda and asked the meeting for some degree of flexibility since the time estimated for several of the agenda items were difficult to foresee. Two new documents were introduced; (i), the discussions from WNT20 on Performance-Based Test Guidelines (PBTG); and, (ii) the draft Test Guideline for Skin Irritation. The agenda was adopted by the meeting and is attached as Annex 1.

OPENING OF THE MEETING

4. The Secretariat explained the background to the establishment of the VMG-NA, and the decision by the 6th Meeting of the EDTA of the Test Guideline Programme in 2002 to start a 3rd VMG based on the great importance of, an urgent need for, relatively cheap and quick high-throughput screens and tests not requiring animals. The VMG-NA was updated on the latest events of the EDTA and the WNT and that there is an ongoing discussion at the WNT of the exact roles of the EDTA and the three VMG's.

5. Participants introduced themselves to the meeting and the list of participants is attached to this report as Annex 2.

PRESENTATIONS

JAPANESE ACTIVITIES

6. Masahiro Takeyoshi presented the Japanese activities since the VMG-NA5 meeting, see meeting document No. 3. See annex 3 for a compilation of the assays that are presently under development by the VMG NA.

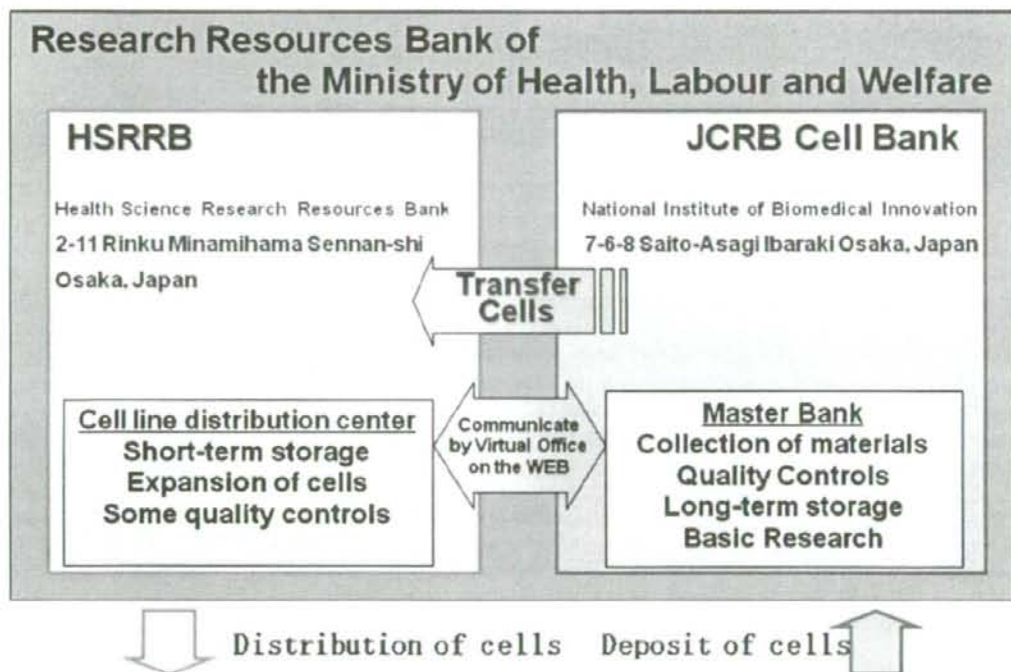
7. Atsushi Ono and Hajime Kojima updated the meeting on the progress with the STTA antagonist validation study. The validation report will be an OECD document available by the end of March 2009 that will be submitted to the WNT. JaCVAM will establish the peer review panel using the newly formed Framework for International Cooperation on Alternative Test Methods (ICATM; [http://www.fda.gov/oia/ICATM.htm]). The Secretariat informed the meeting that there are ongoing

discussions how to formalise the co-operations between the OECD and ICATM. It was discussed whether the antagonist STTA should be a Test Guideline of its own or also include the agonist assay. It was generally agreed that the aim should be to have both assays under the same Test Guideline; the current STTA TG could be revised when the antagonist assay validation study is completed.

8. The Secretariat explained that the circulation of the draft DRP on "Environmental Endocrine Disruptor Screening: The Use of Receptor Binding and Transactivation Assays in Fish" has been delayed but that the Secretariat aims at circulating the draft DRP to the WNT for comments before the end of 2008. A revised version was made available by Japanese Ministry of the Environment already in June 2008, but due to lack of resources, an earlier submission to the WNT has not been possible.

AVAILABILITY OF THE HELA CELL LINE AND INITIAL DISCUSSIONS ON MTA

9. Masahiro Takeyoshi made a presentation how the HeLa cell line was publically available through the Japanese Collection of Research Bio-Resources (JCRB) of the National Institute of Biomedical Innovation. The cell line was developed by Sumitomo Chemicals Ltd who has deposited the cell line at JCRP. The cell line distribution centre is at the Health Science Research Resources Bank (HSRRB), where cells are transferred to from JCRB (See figure below from Masahiro Takeyoshis' presentation). Any user of the cell line needs to sign a Material Transfer Agreement (MTA) with Sumitomo.



10. A long discussion followed on the MTA and the exact text in the MTA and Dan Dietrich raised a warning finger if the text would say 'for research only", since that would not be appropriate for an OECD Test Guideline. The Secretariat agreed and mentioned the criteria in Guidance Document No.34 on the use of patented methods for Test Guidelines. A transparent approach would have to be needed and the cell line would have to be fully available to all users of the STTA OECD Test Guideline. The meeting asked

Masahiro Takeyoshi to try to get hold of the MTA in its present shape and to make a translation. The meeting also agreed that Dan Dietrich should draft a generic text describing what could be in an MTA and the Secretariat also asked the PBTG WG to make an inventory of all elements that could be covered by similar MTAs' in the 17 assays under validation of the VMG NA. The issue was tabled until the following day.

11. Masahiro Takeyoshi presented a provisionally translated version of the MTA, since the official English translation was not possible to get hold of since it was under translation. The meeting discussed the provisional MTA and a number of rather severe comments were raised whether the MTA would actually allow a laboratory to use the cell line under the STTA Test Guideline. The most severe restriction included that the MTA was to be used for research only, which would not be appropriate for an OECD Test Guideline. The bio-safety level mentioned and the exact wording of the text regarding registration purposes were also difficult to fully accept. To resolve the issue, the Secreetariat will stay in contact with Masahiro Takeyoshi to further discuss the English version of the MTA and whether changes will be necessary. If necessary, the Secretariat will also contact the legal services for clarifications.

EU ACTIVITIES

12. Susanne Bremer gave a short update on the progress with the EU ReproTect project and informed the meeting that the MELN, AR ER CALUX are in a statistical evaluation phase and information could not be made available prior to the meeting due to this. There is an ongoing internal discussion in ReproTect Consortium how to proceed and publish the data, and also some questions whether additional chemicals needs to be tested. As soon as the information is available it will be distributed to the Secretariat and the VMG NA. Susanne Bremer informed that some technical problems had been encountered for a laboratory participating in the Japanese antagonist validation study,

METABOLISM

13. The Secretariat informed that the DRP on metabolism has been declassified and adopted and is available on the website. As pointed out in earlier meetings it is important to try to include aspects of metabolism in *in vitro* testing of endocrine disrupters and discussion on metabolism is a standing point on the agenda for the VMG NA.

14. Christine Nelleman gave a presentation on "*Metabolism of S9-fraction and human hepatocytes*". The objective of the study was to compare human S9 fraction with primary human hepatocytes for a range of chemicals. The preliminary conclusions were; (i) human S9 and human primary hepatocytes resulted in the same level of degradation; (ii), the T-screen assay shows no internal metabolism; (iii), the azole fungicides were not metabolised; and (iv), the phthalates and the parabens were metabolised. Future work will include testing of more chemicals and other in vitro assays, however, limitations of the assays included that induction of S9 varies and availability of human S9 can be difficult.

15. Steve Bradbury on the background of the DRP on Metabolism and the interpretations on dosimetry, are there any other activities going on that have a bearing for the DRP. Miriam Jacobs replied that there where three sets of recommendations, short, medium and long term. Rat S9 mix was the recommendation from the DRP, but Christine has jumped directly to human S9 mix and that could be very interesting for future screening studies, but how could one progress? It was obvious that is was not feasible to use human hepatocytes since you only get one shot due the limitation of material. Miriam Jacobs informed the meeting that an ECVAM workshop on Metabolism were held in Ispra, Italy 4-5 September 2008, and the Meeting Report will be made available on the OECD Protected Website. The ECVAM workshop was seeking stakeholder input in the area of metabolism. The workshop participants recommended to validate protocols using HepRG cells and cryo-preserved human hepatocytes. The metabolic competence of the cell

systems is evaluated by assessing the induction of predefined CYPs. The validation of the HepRG cells is performed in the context of the EU project "Vitrocellomics" which is aiming at making metabolic competent systems available including hepatocytes derived from human embryonic stem cells. Human embryonic stem cell derived hepatocytes have not been considered for validation yet because the standardisation of the cells differentiation was not sufficient to enter into formal validation.

US EPA ACTIVITIES

16. Gary Timm gave an update on the US EPA in vitro test method developmental activities (Meeting document #5)(Annex 3). A new Chimpanzee recombinant AR binding assay is under testing in a contract lab in winter/spring 2009. US EPA is looking into whether a retrospective validation of in vitro ER TA (T47D-KBluc) and AR TA (MDK-kb2) assays are possible, by compiling information on assay performance information from various laboratories. Gary Timm presented the status of ToxCast and out of the over 600 ToxCast assays, six assess estrogen, and 5 each are related to androgen and thyroid receptor signalling. Phase IIa and b will be launched in summer 2009. The Tox21, the interagency effort of the NTP, NCGC and EPA to evaluate high-throughput screens by testing a variety of chemicals is under way. Tox21 anticipate to have 6000-7000 chemicals under study at the end of 2009.

PBTG DISCUSSIONS ***(Secretariats note: this issue was discussed during several sessions and the below section is the general outline of this activity as decided by the VMG-NA)***

17. Steve Bradbury introduced the subject and the structure of the discussions. The Secretariat described the present situation and the outcome of the WNT20 and their recommendation to develop a PBTG, however, it should be noted that individual Test guidelines for each assay should be developed in parallell with the PBTG. The PBTG is an issue that the WNT considers important to resolve and that the VMG-NA has been given a special mandate to discuss and to develop a proposal how a PBTG could look like. The goal of the PBTG WG should be to develop this material as a concrete TG proposal including guidance, if found necessary, and present this material to the WNT22 in spring 2010. The Secretariat reminded the VMG-NA of the existing examples of where Performance Standards have been developed at the OECD and that the existing Test Guidelines 430, 431 and 435 and Guidance Document 34 should be consulted, in addition to other documents where Performance Standards are used or proposed, *e.g.*, the draft Skin Irritation TG. The PBTG WG comprise Gary Timm, Steven Bradbury, Alexius Freyberger, Daniel Dietrich, Kate Willett, Susan Laws, Tim Schrader and Miriam Jacobs (chair).

18. Kate Willet gave a presentation on the use of Performance Standards (PS) and their essential elements for validation based on the Guidance Document No.34. Things for consideration in me-to development needing further discussion included:

1. How similar is similar? Crucial in determining essential method elements, likely to be an iterative process

2. How much verification of performance is needed? Repeatability, sensitivity (ability to detect positives), specificity (ability to detect negatives) and accuracy (the proportion of correctly identified chemicals): depends on the quality of data available for the validated assay, for the reference animal test and for the species of interest, which is often human

3. How many and what kind of reference chemicals? Represent the range of responses and range of applicable chemicals

4. How much and what kind of review is necessary? Independent peer review is considered crucial to validation, ad-hoc panel - Single round or multiple rounds? Expedited procedures?

5. How much, if any, stakeholder input is needed? Is essential for initial method validation, but can add up to a year per round of commenting

19. She further presented some examples of what could be included into a PBTG by identifying essential test method components from the four ERα-assays, including T47D, MELN, LUMI and STTA. The LUMI and STTA agonist and antagonist assays should be the basis for the PBTG. Non-variable and variable test method components needs to be determined and four main topics needed further discussion.

1. Essential test method components
   →Non-variable components
   →Variable components that needs definition
2. Minimum list of reference chemicals
3. Accuracy values
4. Degree of peer review needed, stakeholder participation

1. Essential Test Method Components - Non-variable test method components:

- Non-variable components included only stably transfected or endogenous receptor and marker genes (no transient) assays, since Susan explained that transient assays shows a lot of variability and might be difficult to include in the PBTG.

- The Luciferase detection system is the mode of choice, if other detection systems are proposed in the future the scope of the PBTG might have to be revised.

- The Data Interpretation Procedure (DIP) should be the same for all assays, *e.g.*, PC10, PC50 or other like EC 50. Jenny Odum suggested that the criteria for whether a compound is positive or negative needs to be the same for all assays otherwise you cannot compare the different assays. Gary suggested that maybe the common chemicals used for the different assays could be compared and the different DIPs' evaluated. The DIP may be a variable component but that needs to be checked by actual comparisons and the PBTG WG will further discuss this and clarify the situation. Alexius Freyberger pointed out that the DIP needs to be consistent otherwise different assays can give different results, if you for instance end up on the EU ED hit list for further evaluation using an *in vitro* test you may be forced to test further *in vivo*. Steve Bradbury pointed out that if you have two assays that are similar but with different DIPs', they may result in different results, are there any implications on the PBTG in case of that? Alexius Freyberger still expressed doubts about the use of PC10. Patricia Schmieder pointed out that the DIP should identify if anything is significantly different from the control, how do you explain the DIP in that sense? She further asked for models to compare the DIPs' for all used units. Gary Timm agreed that depending on what DIP you are using you may end up with different results, why we need to have the same DIP.

1. Essential Test Method Components - Variable components

Kate Wilett concluded that the STTA PBTG should be for human ERα only because you are anticipated to get different responses in different species, probably no uniformity across different phyla. Steve Bradbury reiterated the exact definition of a screen, as read in GD34 and asked that the exact role of the PBTG needs to be clarified together with it's regulatory purpose and contribution to hazard assessment. Dan Dietrich clarified that the purpose of the assay is to screen for binding to ERα and the exact regulatory use may be variable. The Secretariat concluded that this is a reoccurring issue that is always brought up for discussion at the VMG NA and that there is an inherited problem for the development and validation of our assays, since we do not explicitly have a description of the regulatory use of our methods. An OECD meeting will be held in September 2009 in Denmark to start looking at proposed uses of the ED test methods, but not to revise the conceptual framework.

The variable components included:
- Cell line
- Characteristics of cell line including metabolism
- Culture conditions (media, pH, etc.)
- Plating density
- Plate layout
- ER characteristics
  - Full length or partial
  - Species of receptor
- Marker gene construct
  - Promoter, receptor binding elements, reporter
  - CERI STTA uses mouse metallothionin promoter + 5 tandem VTG ER binding sites + luciferase
- Method of determining cytotoxicity

2. Minimum List of Reference Chemicals
The meeting agreed that 2-4 control chemicals, 12 proficiency chemicals and 40-50 reference chemicals would be adequate.

H295R Steroidogenesis Assay

20. Markus Hecker presented the validation work on the H295R Steroidogenesis assay. The phase 2 of the validation study has been completed and the peer review is expected within a couple of months after the VMG NA. The H295R Steroidogenesis Assay is a rapid, economic, reliable, and cost effective screen of chemicals for their potential to alter Steroidogenesis (priority setting, Level 2 screening). Data suggest excellent transferability and relevance of the assay. The 2 main issues brought up by Marcus Hecker were (i) Issue on the viability measurements, using the Live/Dead and the MTT protocol; and (ii), what type of assessment desired (LOEC, EC50 or PC) should be used and whether quantitative versus semi-quantitative or categorical should be used? After some discussions, the meeting recommended that performance criteria for one of the cell viability measurements could be introduced into the test method, giving the opportunity to use either of the methods, providing you can justify the choice. US-ICCVAM has produced a document on Cell Viability Measurements that could be consulted.

21. A discussion on cross-reactivity followed; is there a need to take out the two top concentrations to reduce the cross reactivity, would that result in too few remaining concentrations? If we have overlap in cross reactivity at the highest two concentrations you probably need to retest in another system. The recommendation from the meeting was to stay with the existing protocol for now and finalise the validation study. Exactly how next step regarding the issue on cross reactivity will be handled can possibly wait until VMG NA7, or if necessary, the Steroidogenesis ad hoc expert group (Gary Timm (chair), Markus Hecker, Miriam Jacobs, Alexius Freyberger, Christine Nelleman, Tim Schrader and Daniel; Dietrich) might be initiated at an earlier occasion if found necessary. After some consultation Gary Timm updated the meeting on the timelines for the development of the assay;
- December 2008, dealing with outstanding issues
- January 2009, validation report
- February, review of the validation report by the ad hoc sterodogenesis group and the EPA study group. Ammend as apropriate
- April, peer review
- June 2009: submit draft peerr eview report and draft Test Guidleine to VMG NA.

The US Recombinant ER-Binding Assay

22. Shirlee Tan presented the validation plan for the recombinant ER-binding assay over the phone. The validation sudy is a joint effort between US EPA, EC-ECVAM, CERI/METI and Bayer Healthcare. Two protocols (FWA and CERI) are being evaluated and the final testing is expected to be concluded by the 2nd quarter of 2009. The data will be analysed at the US EPA's Data Coordination Center and subjected to peer review probably in early 2010. Steve Bradbury wondered about the classification scheme, and Shirlee Tan explained that over 50% would be classified as a binder, under 25% is negative and between 25-50% equivocal.

The LUMI-CELL ER TA Validation Study

23. Ray Tice presented the international validation effort of the LUMICell assay over the phone, with US-NICEATM, EC-ECVAM and JaCVAM. The largest problem with the studies done so far is the variability of the plate acceptance rate. The plate acceptance criteria have been set pretty tight and with some modifications, based on the results of the qualitative and quantitative comparisons, considerably better results were received. Actual testing is expected to be finalised by February 2009, a Background Review Document will be available in summer 2009 and the peer review and presentation of the Peer Review Report is expected in Fall 2009.

QSAR Discussions

24. Patricia Schmieder presented the work of the ED QSAR task group and the latest developments by US EPA, EU-IHCP/EFSA [Available: http://ecb.jrc.ec.europa.eu/qsar/information-sources/], and Japanese NIHS. Planned activities for 2009 included: (i) Participate in OECD Expert Consultation on Scientific and Regulatory Evaluation of a QSAR Approach for Estimating Estrogen Receptor Binding Affinity for Chemicals in Defined Regulatory Inventories; and, (ii) Start dialogue with OECD QSAR Assessment Toolbox Management Group on QSARs/prediction systems for metabolism (ED and other)

25. Bob Diderich from the Secretariat presented the QSAR Application Toolbox and the planned QSAR Workshop on Scientific and Regulatory Evaluation of a QSAR Approach for Estimating Estrogen Receptor Binding Affinity for Chemicals in Defined Regulatory Inventories, to be held 17 February 2009, in Paris. The purpose is to review the science (VMG NA) and improve regulatory acceptance to include a number of new profilers for the QSAR ToolBox. The tool is applicable for any type of regulatory applicability where chemical grouping is allowed, *e.g.*, REACH as one preferred data gap filling activity. EC-ECHA is financing phase 2 of the project.

CONCLUDING REMARKS AND COMITTMENTS

H295R STEROIDOGENESIS ad hoc GROUP

26. The Sterodogenesis ad hoc Group will convene as found necessary to assist in the development of the H295R draft Test Guideline and the validation/peer rveiew process.

PBTG

27. A definite set of reference chemicals that are the same for the LUMI-CELL and the STTA should be defined and then used to challenge the draft PBTG with the other two test methods, as they become available. Draft TG will be prepared by combining earlier generic drafts with Ray Tice's comments from the LUMI-CELL method (Miriam Jacobs, directly after the meeting). A contractor will be hired by US EPA to extract essential test method components and prepare draft chemicals list and specific performance

requirements, results should be ready by the end of the 1st quarter of 2009. The PBTG WG and other invited experts will combine the draft TG plus results from contractor to create a draft PBTG for the next VMG-NA. A question that remains to be discussed is how to handle the protocols (in a Guidance Document?). Comments plus final draft PBTG to be prepared for WNT 22 in 2010.

MTA Issue

28. The Secretariat will follow up on the MTA issue and contact Masahiro Takeyoshi regarding the English MTA version. An MTA group was established, comprising Susan Laws (chair), Gary Timm, Daniel Dietrich, Steven Bradbury and Miriam Jacobs that will look into whether other assays under development in the VMG-NA also have elements that will be covered by MTA. The group will report to the Secretariat before the end of 2009.

29. The Secretariat thanked all participants and the two co-chairs for a successful meeting. The meeting report will be circulated shortly after the meeting for a short turn-over time and the List of Participants are to be circulated to members of the VMG NA. The VMG-NA7 is planned to be hosted by US EPA in some nice meeting venue sometime in October-December 2009.

Paris, 12 January 2009
Patric.Amcoff@oecd.org

ANNEX 1
List of participants
(only available to govermental representatives)

ANNEX 2

6TH MEETING OF THE VALIDATION MANAGEMENT GROUP FOR NON-ANIMAL TESTING (VMG-NA)
19-21 November 2008, OECD Paris

DRAFT AGENDA (Rev 2)

Tuesday 18 November

The meeting of the STTA sub-committee and the QSAR working group will take place at the Environment Directorate at Porte Maillot, 15 boulevard de l'Amiral Bruix. The QSAR group meet at 9-13 and the STTA group at between 14-18, in the meeting room on third floor.

Wednesday 19 November

09h00-09h10	**Opening of the Meeting, Explanation of OECD Procedures** - There will be a brief explanation of the role of the members, the status of documents and other general procedures. Listing of Meeting documents.	
09h10-09h20	**Approval of the Draft Agenda**	
09h20-09h30	**Introduction of the Membership of the VMG-NA**	
09h30-09h40	**Introduction of the Special Activity on ED Testing by the Test Guidelines Programme** - There will be a brief summary of the history of the VMG's and the EDTA (Secretariat).	Meeting documents 1, 2
09h40-10h00	**Update on activities by Japanese CERI, METI, NIHS, MHLW and MOE** - General presentations of activities (Presenter to be confirmed!) - The Draft Fish In Vitro Receptor DRP (Secretariat)	Meeting document 3
10h00-10h45	**Update on the Japanese STTA Validation Study** - Presentations by Hajime Kojima - Presentation by Masahiro Takeyoshi	Meeting document 4
10h45-11h15	**COFFEE/TEA BREAK**	
11h15-12h00	**Update on ECVAM activities** - General presentations of activities (Presenter to be confirmed!)	

12h00-12h30	**Issues on Metabolism** - Update by the Secretariat on the DRP - Presentation by Christine Nellemann on "Metabolism of S9 fraction and human hepatocytes"	
12h30-13h45	**LUNCH BREAK**	
13h45-14h15	**Update on US EPA activities** - Presentation by Gary Timm	Meeting document 5
14h15-15h00	**Initial discussions on PBTG's** - Presentation by the Secretariat on the use of Performance Standards - Presentation by the EC on the use of Performance Standards (presenter to be confirmed)	Meeting documents 1, 2, 6-11
15h00-15h30	**COFFEE/TEA BREAK**	
15h30-17h30	**Continued discussions on PBTG's** - Presentation of the work by the STTA sub-committee by Miriam Jacobs (EC)	Meeting documents 1, 2, 6-11
17h30	**ADJOURN FOR THE DAY** ***- The STTA validation group will meet in room CH 320 or CH 331 at between 18-20.***	
Thursday 20 November		
09h00-09h05	**Review Day 1 and Objectives for Day 2 (Secretariat)**	
09h05-10h30	**The H295R Validation Study Plan** - Presentation by Marcus Hecker (US EPA) - Discussions	
10h30-11h00	**COFFEE/TEA BREAK**	
11h00-12h30	**Continued discussions on PBTG's** - Presentation of the work by the STTA sub-committee by Miriam Jacobs (EC)	
12h30-13h45	**LUNCH BREAK**	
13h45-14h45	**The Validation Plan for the Human Recombinant ER-Binding Assay** - Presentation by Shirlee Tan (US EPA), over the phone - Discussions	
14h45-15h00	**COFFEE/TEA BREAK**	

15h00-15h40	**The LumiCell Pre-validation Report** - Presentation by Ray Tice (US NICEATM), over the phone - Discussions	
15h40-16h00	**Presentation on Progress of the QSAR Group** - Presentation by Pat Schmieder (US EPA) - Discussions	
16h00-17h00	**Presentation of the OECD QSAR Tool Box by Bob Didrich (Secretariat)** - Discussions	Meeting document 12
17h00-17h30	**AOB**	
Friday 21 November		
09h00-09h05	**Review Day 2 and Objectives for Day 3**	
09h05-11h00	**Continued Discussions on the PBTG's** - Discussions - Work plans, establishment of sub-groups, dead lines, etc,	Meeting documents 1, 2, 6-11
11h00-11h30	COFFEE/TEA BREAK	
11h30-12h00	**Any Other Business**	
12h00-13h00	**Concluding Discussions Time Frames and Commitments for Activities**	
13h00	**MEETING ADJOURNED**	

Meeting Documents

Documents are available on the protected website:
http://webdomino1.oecd.org/comnet/env/tf-edta.nsf?OpenDatabase
User name <<more>>, Pass word <<estrogen>>; and then go to VMG NA5

Meeting Document #1	Meeting Report from EDTA 11, 1-2 April 2008 [ENV/JM/TG/EDTA/M(2008)2]
Meeting Document #2	Meeting Report from the 5th Meeting of the VMG-NA
Meeting Document #3	Update Validation Status of Non-animal Testing in Japan, CERI (Chemicals Evaluation and Research Institute), Japan, METI (Ministry of Economy, Trade and Industry), Japan, NIHS (National Institute of Health Science), Japan, MHLW (Ministry of Health, Labor and Welfare), Japan, MOE (Ministry of the Environment), Japan
Meeting Document #4	How to obtain the HeLa cell line
Meeting Document #5	Update on US EPA's In Vitro Method Development Activities

Meeting Document #6	Guidance Document No. 34 on the Validation and International Acceptance of New or Upgraded Test Methods for Hazard Assessments
Meeting Document #7	In vitro test methods: Performance based Test Guidelines (PBTG) and mee to[ENV/JM/TG(2008)33]
Meeting Document #8	Test Guidelines 435 on In Vitro Membrane Test for Corrosivity Testing
Meeting Document #9	Test Guidelines 430 "EPISKIN"
Meeting Document #10	Test Guidelines 431 "TER"
Meeting Document #11	11.1 Revised STTA TG 11.2 How to use the spreadsheet 11.3 Spreadsheet for example calculations
Meeting Document #12	Outline for ER binding WS

ANNEX 3.
MAIN ONGOING PROJECTS AND THEIR VALIDATION STATUS

Receptor Binding Assays				
hrERα	*Protocol 1.* The FWA assay protocol utilizes the Pan Vera hrERα full length ER. *Protocol 2.* CERI protocol utilizes the CERI-ERα, which contains the ligand binding domain of hrERα.	binding	Validation starting in early 2008. Peer review by 2nd quarter of 2009. SPSF submitted.	US lead international collaboration study
rAR	Human recombinant AR assay. Ligand binding domain expressed in E. coli.	binding	Under development. Approximately 900 chemicals have been tested.	METI
	Rat recombinant AR assay (ligand binding domain is identical to human LBD).	binding	Some limited prevalidation work has been conducted in the EU project ReProTect. Options are currently being explored.	Lead and international collaboration work under discussion
hrTR	Human recombinant TR assay. Full-length expressed in E. coli. TRs α1 and β1 binding assays.	binding	Under development. Approximately 60 chemicals have been tested using both receptors.	METI
Transcriptional Activation Assays				
ERα	HeLa-9903 cells with plasmids containing hERα cDNA driven by SV40 promotor and luciferase reporter plasmid. STTA.	Stable, ag/antag	Provisionally approved by WNT20 for agonist part. Antagonist assay validation report in March 2009.	CERI/MHLW
	HeLa-9903 cells: hERα/pcDNA3.1 receptor expressing plasmid and ERE-AUG-Luc+ reporter plasmid	Transient, ag	Validated under domestic multi-lab. using same test chemicals as hERa-HeLa-9903 cell line. SPSF submitted. Peer review in 2nd quarter of 2009.	CERI/MHLW
	MELN. MCF-7 cells with endogenous ERα + luciferase stably transfected	ag/antag	Validation in 2008, presentation of data in 1st quarter of 2009.	EC/ECVAM