Introduction

1. This meeting was held three years after the fourth VMG-mammalian meeting (April 2003). The main objective of the meeting was to review the status of the work with respect to projects included in the rolling work plan of the Test Guidelines Programme, and to agree on the appropriate next steps.

Agenda Item 1: Opening of the meeting, Introduction of participant

2. Mike Wade (Canada), Chair of the VMG-mammalian, invited all participants to introduce themselves (see Annex 1 for the Participants List).

Agenda Item 2: Approval of the Draft Agenda

3. The agenda [document ENV/JM/TG/EDTA/A(2006)1] was approved with the addition of a discussion on chemical repository under "Other issues".

Agenda Item 3: Need for further work in Parallel with the development of the Test Guideline for the Uterotrophic Bioassay

4. Peter Gelbke, consultant for the Secretariat, summarized the peer review panel report, as well as the peer review panel general agreements and major concerns. The peer review panel was especially concerned that only one oestrogen antagonist, one negative substance and one substance with enhanced activity due to metabolism were tested.

5. Peter Gelbke then presented a proposal for a simple retrospective validation with respect to one or more negative substances. On the basis of negative results in a 2-generation test, an uterotrophic assay, an ER binding assay and a E-Screen, he had identified styrene as a possible candidate, noting that some points are yet to be checked in the 2-generation study (in particular the developmental neuro-toxicity). In addition he proposed that the data provided by Japan with the Uterotrophic Bioassay and two in vitro screening tests (hER receptor binding assay and hER-alpha reporter gene assay) should also be used as support for the specificity of the Uterotrophic Bioassay.

6. Gary Timm informed the VMG of the recommendations of the EPA' Endocrine Disrupter Methods Validation Advisory Committee (EDMVAC) that the EPA support the development of a TG only with regard to the use of the assay for estrogenic chemicals.

7. The VMG agreed that the most important was to identify one or two additional negative substances. For oestrogen antagonist, Japan may be able to provide a report with existing data to show the specificity. Experience with Test Guidelines would provide information on substances with enhanced activity due to metabolism.

8. After a long discussion about what the criteria for a "negative" substance should be, the VMG agreed that the acceleration of vaginal opening is a valuable criterion to consider in addition to increased uterine weight in immature females. It also agreed that styrene might be a good candidate. In addition, a review of the results of (i) uterotrophic assays and (ii) 1 or 2-generation assays carried out in Japan might provide one or more other negative substances. Peter Gelbke will review the data provided by Japan and prepare a paper, if possible for the EDTA meeting.

Agenda Item 4: Development of the new Test Guideline for the Uterotrophic Assay

9. Peter Gelbke presented the Draft Test Guideline. The VMG agreed that Jun Kanno will check whether the text that is already included in the proposed TG with respect to mice use is sufficient and he

will provide a report supporting the use of mice. The TG might be revised at a later stage on the basis of this report.

10. The VMG provided advice on a number of issues for which specific guidance might be necessary. In particular, the VMG agreed that there was no need to specify criteria for negative versus positive, or weak versus strong estrogens, and recommended that the TG should contain guidance on oestrogen content in the feed and on control uterine weights in immature females.

11. Peter Gelbke will revise the draft on the basis of the comments made by the VMG and include the testing for anti estrogenic effect in an appendix as an option. This will be done in consultation with Japan, which is the lead country, with the Secretariat, for this project.

Agenda Item 5: Background Review Document to support the Hershberger Assay

12. The Background Review Document was not available for the meeting; therefore it was not discussed. However, the VMG took note of the status of validation work and discussed how to finalize it.

13. First, Willie Owens presented a status report on the Phase 3 validation of the assay with the castrate model (determination of inter- and intra-laboratory variability and laboratories blinded to identity of test chemicals). The VMG agreed that the Hershberger bioassay is valid for androgen receptor agonists and antagonists and appears feasible for 5α-reductase inhibitors. The VMG took note of the fact that for antagonists, the co-administration of two compounds results in baseline variability, and that only one potent 5α-reductase inhibitor was tested; however, no more validation work was considered necessary for the castrate model.

14. Willie Owens then presented a status report on the validation of the assay with the weanling model. It appears that the weanling model may be just slightly less sensitive than the castrate with anti-androgens.

15. The VMG agreed on the following next steps regarding the validation of the assay with the castrate model: (i) drafting of the Background Review Document, (ii) drafting of the Phase 3 validation report (iii) peer review of the assay. For the assay with the weanling model, 6 laboratories are ready to start Phase 3 validation to evaluate the laboratory performance when the identity of the tested chemical is blinded. The VMG recommended that Phase 3 validation focus on antagonists and include two negative substances. Laboratories previously not involved in the work on the weanling model should run a pre-study before entering coded testing. The minimum requested by the VMG was response to TP and antagonism by flutamide. A validation report needs to be developed for the validation of the assay with the weanling model.

16. In order not to delay the development of a TG for the castrate, it was suggested to have two different Test Guidelines (like TG 203A and TG 203B). Whether a light peer review could be sufficient for the weanling will have to be discussed. The Secretariat referred to Document INF 17 and observed that the development of the Test Guideline is not yet included in the rolling work plan and there is no lead country for this activity.

Agenda Item 6 and 8: Validation of the enhanced TG 407 and Draft Test Guideline for the enhanced TG 407

17. Given the low sensitivity of the assay for identifying weakly estrogenic and anti-androgenic substances, the VMG discussed at length the added value of the assay. Different views were expressed. Some participants considered that the enhanced TG would provide additional information at an acceptable additional cost (estimated to be around 10% including thyroid function-related hormone work); other were

concerned that the results of the TG could be misinterpreted and misused, discouraging the use of other tests. The VMG agreed that the enhanced TG 407 should not be considered as an endocrine disrupters screen and should not be used as a substitute of the Uterotrophic or Hershberger Assays. Negative results of the enhanced 407, with respect to (anti)estrogenic or (anti)androgenic modes of action, should not indicate that there is no need for further investigations concerning potential (anti)estrogenic or (anti)androgenic activity. However, considering that (i) the test method is intended to be a general toxicity assay to screen a wide variety of effects rather than a specific mechanism of action (ii) the test method will be used frequently and (iii) it is better to be informed than not informed of any test positive results, the VMG finally agreed on the recommendation to develop an updated Test Guideline that would replace the current one, subject to the insertion in the Test Guideline of a clear text describing the TG limitations. This text is included in Annex 2.

18. The VMG also agreed on the proposed changes to the Draft final report as included on Document INF 13. The report will be further revised to reflect the above discussion and to include corrections sent by the US.

19. There is no project in the rolling work plan for the development of the updated TG 407 and no lead country; therefore, according to the recent agreement on how to include a project in the Test Guidelines rolling work plan, a lead country or the European Commission should submit a proposal.

20. If the decision to update the Test Guideline is taken, the VMG recommends to convene an international group of pathologists to give detailed guidance on (i) interpretation of subtle hormone changes in target tissue without frank toxicity [for hormone dependant tissues, (male) mammary gland and oestrous synchronisation of female reproductive organs] (ii) dissection procedures for critical tissues, and (iii) evaluation of vaginal smears). Such guidance would improve the assay power and sensitivity. The VMG discussed whether T3, T4 and TSH hormones should be measured routinely and agreed that measurement should be triggered by thyroid histopathology.

21. The VMG recommended that the EDTA Task Force discussed the feasibility of establishing an inventory for all tests carried out according to the updated TG.

Agenda Item 7: Information on peer review process

22. Gary Timm presented the meeting document MD4 that includes the proposal to manage peer review by contract. From experience with the peer review of the Uterotrophic Assay validation, it appears that it is very important that the panel members first agree on what the validation principles should be for the *in vivo* or *in vitro* assay. Expertise should be the most important criteria for selecting the 6 to 10 panel members and a laboratory representative should be available to respond to the panel member questions. All data, and decision rationales related to the validation should be provided to the peer review panel.

Agenda Item 9: Detailed Review Paper (DRP) on Thyroid Hormone Disruption Assay

23. Shirlee Tan presented the DRP. The Secretariat indicated that the DRP would be posted on the EDTA Website for the end of April meeting.

Agenda Item 10: Level 5 Studies

24. Several presentations were made on activities related to the enhancement/improvement of reproductive toxicity tests, aiming to replace the 2 generational toxicity test (OECD TG 416). Based on an extensive database of 2-generation studies, it appears that examination of the F2 generation does not provide increased sensitivity (compared to examination of F1 animals) at detecting the effects of endocrine

disrupters. Furthermore, the sacrifice of F1 and F2 at very young ages prevents the detection of late onset pathology that comes with pathology senescence.

25. James Lamb (US) presented the ILSI/HESI project "The Life stages F1-extended one-generation development and reproductive test to develop an alternative test to the current or standard two-generation reproductive toxicity test". The test is designed as a tiered approach and would evaluate toxicity to reproductive, neural and immune system development. It would greatly reduce the number of animals required for detailed testing of pesticides compared to current requirements.

26. Dr. Aoyama (Japan) presented a project of the Japanese Ministry of Environment "The Enhanced one-generation reproductive toxicity study in rats for predicting low-dose effects". In addition to classical endpoints, molecular responses, in particular expression of estrogens, androgen receptors and ER- and AR responsive genes in the uterus and prostate gland were also evaluated.

27. Jun Kanno (Japan) presented a research project on a definitive testing method "The Rodent one life-span test", which is part of Screening and Testing Scheme for endocrine disrupting chemicals, adopted by the Japanese Ministry of Health Labour and Welfare. Based on the knowledge that traditional one-gen and two-gen protocols are not designed for the detection of receptor-mediated toxicity/low dose effects, the objective of this approach is to capture low dose effects that can be declared "adverse" by evaluating specific endpoints, such as the early onset of persistent oestrus in female mice; therefore, the work is more directed to developing new protocols than rearranging or modifying pre-existing protocols so that it is less advanced in terms of proposing methods/strategies. This activity may lead to a proposal that is different from the proposal made by the US and it was suggested that the outcome of the work on real low doses adverse effect would influence the US proposal at a later stage.

28. The VMG took note of the current work, and agreed to wait for the US retrospective analysis before discussing what the role of the VMG could be with respect to the development of a new or enhanced test, considering that a presentation on the life-steps test was already made at the OECD Working Group on Pesticide.

Agenda Item 11: Intact Male Assay

29. D. Bergfelt (US) and J. O'Connor (BIAC) presented progress with the validation of the 15-day intact adult male rat assay. The VMG took note and welcomed the development of this test. It noted the differences with the enhanced 407, in particular in terms of the number of animals (no less than 15 per group) and the capability of the laboratories to try and control for extraneous factors (e.g. method and time of blood collection and animal stress) that may affect the various hormone concentrations determined in this assay.

Agenda Item 12: Declassification of validation reports and peer review panel reports

30. The VMG supported the declassification of the reports. For the report of the peer review panel, it recommended to keep it anonym. The validation report of the enhanced TG 407 should be checked to ensure that all laboratory names have been removed.

Agenda Item 13: Other issues

31. The VMG was informed that the chemical repository, at the TNO, would be closed after Phase 3 validation of the Hershberger Assay using weanlings. Some countries or the other VMGs might be interested by the remaining chemicals. A proposal was made that the TNO should list the amounts of chemicals still available including the expiration dates. The VMG recommended that this issue be discussed at the EDTA Task Force meeting.

ANNEX 1

List of Participants

Meeting of the Fifth Validation Management Group for Mammalian Testing
Washington

4 April 2006 - 5 April 2006

Available to Government representatives only

ANNEX 2

Initial Considerations and limitations

The TG 407 has been modified to include endpoints to identify chemicals that interfere with thyroid physiology and affect the male and/or female reproductive organs in young adult animals, while still investigating all other toxicological parameters required under the prior TG 407. On the basis of data generated in the validation process, it must be emphasized that the sensitivity of this assay is not sufficient to identify all substances with (anti)androgenic or (anti)estrogenic modes of action. Consequently, the absence of effects in these endpoints can not be taken as evidence for the lack of such effects.

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Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

19-Apr-2006

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

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Task Force on Endocrine Disrupters Testing and Assessment (EDTA) of the Test Guidelines Programme

DRAFT AGENDA OF THE MEETING OF THE TASK FORCE ON ENDOCRINE DISRUPTERS TESTING AND ASSESSMENT

26-27 April 2006, Sundbyberg, Sweden

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English - Or. English

9th Meeting of the Task Force on Endocrine Disrupters Testing and Assessment, Sweden, 26-27 April 2006

Draft Agenda

Wednesday 26 April 2006			
09h00	**1**	**Opening of the meeting by Mike Wader and Ethel Forsberg, Introduction of participants**	
09h15	**2**	**Adoption of the Draft Agenda** The reports of the three VMG meetings are provided as background documents.	**ENV/JM/TG/EDTA/A(2006)2**
09h20	**3**	• **Confirmation of the approval of the summary report of the 8th EDTA Task Force meeting** The Task Force will be invited to confirm the approval of the Summary Record of the 8th meeting. • **Information by the Secretariat** The Secretariat will report on issues related to the Test Guidelines Programme, which were discussed or agreed by the WNT and the Joint Meeting since the last EDTA Task Force Meeting.	**ENV/JM/TG/EDTA/M(2005)1** **INF. 1** **ENV/JM(2006)7**
	4	**Follow-up to the VMG-mammalian**	**Meeting Document 3**
09h30	**4a**	**Uterotrophic bioassay** The Chair of the VMG-mammalian (or the Secretariat) will report on the outcome of the discussions at the VMG Meeting; Peter Gelbke will present a retrospective study on negative substances and the draft Test Guideline. The Task Force will be invited to provide comments.	**ENV/JM/TG/EDTA(2006)9** **ENV/JM/TG/EDTA(2006)12**
10h15	**4b**	**Hershberger bioassay** The Chair of the VMG-mammalian (or the Secretariat) will report on the outcome of the VMG meeting. The Task Force will be invited to agree on a timeline for the finalization of the validation reports of the Hershberger bioassay (castrate and weanling models). The VMG proposal on how to proceed with the peer review of the castrate model validation will be also discussed under Item 5c. An SPSF should be submitted by a lead country or the EC for this project.	

10h30	*Coffee Break*		
11h00	**4c**	**Enhanced TG 407** The Secretariat will present the outcome of the VMG-mammalian discussions (including the possibility to create an inventory of tests results) and present the changes made to the validation report and the draft Test Guideline. The Task Force will be invited to endorse the validation report and comment on the draft Test Guideline and on the possible inventory. The VMG proposal on how to proceed with the Peer Review will also be discussed under Item 5c.	**Meeting Document 1** **Meeting Document 2** **INF. 9** **ENV/JM/TG/EDTA(2006)10**
11h30	**4d**	**Level 5 studies** The Chair of the VMG (or the Secretariat) will report on the VMG discussion. After the VMG meeting, a preliminary proposal (informal SPSF) was submitted by the US (INF 13). The EDTA will be invited to comment and make a recommendation on how to proceed with this issue.	**INF. 10** **INF. 11** **INF. 12** **INF. 13** **INF. 14**
12h00	*Lunch Break*		
	5	**Common issues to all VMGs activities**	
14h00	**5a**	**Detailed Review Paper on Thyroid Hormone Disruption Assays** The US will present the DRP. The Task Force will be invited to comment on the DRP for which WNT approval will be requested.	**Meeting Document 4** **Meeting Document 5** **Meeting Document 6**
14h30	**5b**	**Role and Utility of the Amphibian Metamorphosis Assay** Germany, co-lead country with the US and Japan, will present Document ENV/JM/TG/EDTA(2006)2.	**INF. 3** **INF. 4** **ENV/JM/TG/EDTA(2006)2**
15h00	**5c**	• **Issues related to validation** Gary Timm will present document INF 7. The Secretariat will introduce the short document ENV/JM/TG/EDTA(2006)13. The Task Force will be invited to discuss and consider (i) whether it is possible to develop guidance on how to apply flexibility when implementing DG 34, and (ii) how to develop such guidance. • **Peer Review Process for ED-related assays** The Task Force will be invited to discuss and comment on processes for peer review and on a strategy for the	**INF. 7** **ENV/JM/TG/EDTA(2006)13** **ENV/JM/TG/EDTA(2006)3**

		coming years. The discussion will be finalized on the second day if necessary. (See also Agenda items 4b, 4c, 6a and 7b)	
16h30	*Coffee Break*		
17h00	**5d**	**Chemical Selection** Gary Timm will present Document ENV/JM/TG/EDTA(2006)11 and the Task Force will be invited to address the questions included in Paragraph 3 of the document.	**ENV/JM/TG/EDTA(2006)11**
17h30	**5e**	**Scope of the Detailed Review Paper on In Vitro Fish Assays** This issue will be presented by Japan (or the Secretariat). The Task Force will be invited to endorse the scope of the document.	**INF. 15**
18h00	*Meeting adjourns for the day*		
Thursday 27 April			
	6	**Follow-up to the VMG-eco**	**ENV/JM/TG/EDTA/M(2005)4**
09h00	**6a**	**21-day Fish Screening Assay: peer-review and TG development** The Secretariat will introduce the issue. Japan will propose the action plan and timelines. The Task Force will be invited to endorse the VMG recommendation to proceed to peer-review and TG development (in parallel).	**ENV/JM/TG/EDTA(2006)4** **ENV/JM/TG/EDTA(2006)3** **INF. 5** **INF. 6** **INF. 8** **INF. 17**
10h00	**6b**	**Fish Sexual Development Test: current status** Denmark will provide a short status report. This is for information and discussion.	**ENV/JM/TG/EDTA(2006)5**
10h30	*Coffee Break*		
11h00	**6c**	**Fish Full-Life-Cycle and 2-Generation Test** The US or Japan will present the comparison study on medaka. This is for information.	
11h10	**6d**	**Validation of the Amphibian Metamorphosis Assay** The Task Force will be invited to provide advice on further work that might be needed for the validation of the assay.	**ENV/JM/TG/EDTA(2006)6**

11H30	**6e**	**Validation for invertebrates tests** The Secretariat will present a status report on this activity.	**ENV/JM/TG/EDTA/M(2005)4**
12h00	*Lunch Break*		
	7	**Follow-up to the VMG-non animal**	**Meeting Document 8**
14h00	**7a**	**Draft DRP on the Use of Metabolizing Systems for In Vitro Testing of Endocrine Disrupters** Walter Janssens (Belgium) will present an update on the scope, the recommendations and the comments received. The Task Force will be invited to take note of the DRP current status.	**Meeting Document 7**
14H30	**7b**	**ER Stably Transfected Assay** This test method was developed and validated by Japan. The Secretariat will present an update of the preliminary validation assessment of the assay. The Task Force will be invited to agree on a strategy regarding independent scientific review (the strategy should be proposed by Japan).	**INF. 2** **INF. 16**
15h00	*Coffee Break*		
15h30	**8**	**Joint Meeting Declassification of validation and Peer Review reports** The Secretariat will introduce this issue. The Task Force will be invited to take note of the status of the reports and to provide comments on the document as appropriate.	**Meeting Document 9** **Reports posted on the EDTA Website, under "Reports"**
16h00	**9**	**Peer Review Process (continued)**	
16h30	**10**	**Any other issues** Location and dates of the next VMG meetings (Japan for the VMG - non animal in December, Spain for the VMG-Eco in November, Slovenia for the VMG-mammalian in January?)	
17h00	*Meeting adjourns*		

List of Documents

Meeting Documents		
Item	**Title**	**Reference**
2	Draft Agenda (Revised 19 April)	ENV/JM/TG/EDTA/A(2006)2
3	Draft Summary Record of the 8th Meeting of the EDTA Task Force	ENV/JM/TG/EDTA/M(2005)1
4	Follow-up to the meeting of the VMG-Mammalian (Draft Summary Record)	MD3
4a	Draft Test Guideline on the Uterotrophic Bioassay in Rodents	ENV/JM/TG/EDTA(2006)9
4a	Additional data on the specificity of the Uterotrophic Bioassay	ENV/JM/TG/EDTA(2006)12
4c	Draft Validation Report of Enhanced Test Guideline 407	MD1, MD2
4c	Draft Enhanced Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents; Updated with Parameters for Endocrine Effects	ENV/JM/TG/EDTA(2006)10
5a	Draft Detailed Review Paper on Thyroid Hormone Disruption Assays	MD4
5a	Appendix B - Draft Report on Comparison of Thyroid Activity Measures Across Datasets	MD5
5a	Comments and responses on Draft Detailed Review Paper on Thyroid Hormone Disruption Assays	MD6
5b	Role and utility of various assays related to the detection of thyroid active substances	ENV/JM/TG/EDTA(2006)2
5c	Discussion paper related to the validation of test methods and how flexibility could be applied	ENV/JM/TG/EDTA(2006)13
5c, 6a	Proposed approaches to peer-reviews of validated test methods	ENV/JM/TG/EDTA(2006)3
5d	OECD Reference Chemical Selection	ENV/JM/TG/EDTA(2006)11
6	Follow-up to the meeting of the VMG-Eco	ENV/JM/TG/EDTA/M(2005)4
6a	Draft Paper: Example of application of Guidance Document 34 validation criteria	ENV/JM/TG/EDTA(2006)4
6b	Fish Sexual Development Test: Current Status	ENV/JM/TG/EDTA(2006)5
6d	Validation of the Amphibian Metamorphosis Assay	ENV/JM/TG/EDTA(2006)6
7	Follow-up to the meeting of the VMG-Non-Animal	MD8
7a	Draft DRP on the Use of Metabolising Systems for In Vitro Testing of Endocrine Disrupters	MD7
8	Status of the Validation and Peer Review Reports	MD9
Information Documents		
3	Information on the Test Guidelines Programme	INF.1
3	Report from the WNT on refocusing the Test Guidelines Programme, including a revised Workplan	ENV/JM(2006)7
4c	Changes to the Draft Final Report of the Validation of the Updated Test Guideline 407 Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats	INF.9
4d	A Mammalian Life-Stages Generational Development and	INF.10

	Reproductive Test - An Alternative to the Current Two-Generation Test for Detecting Endocrine Disruptor Effects	
4d	Results of One-generation Tests in Evaluation of the Endocrine Disrupting Activities in Rodents	INF.11
4d	English Draft Report of Screening and Testing Scheme for Endocrine Disrupting Chemicals (MHLW) updated for OECD VMG mammalian, April 4-5, 2006 @D.C.	INF.12
4d	SPSF for a new mammalian level 5 test involving various life stages, submitted by Gary Timm and Don Bergfelt, US EPA	INF.13
4d	A Tiered Approach to Life Stages Testing for Agricultural Chemical Safety Assessment	INF.14
5b	SPSF for a new Test Guideline on Endocrine Disrupters Frog Screen and Test, submitted by the US, August 2000	INF.3
5b	BIAC Letter on Frog Metamorphosis Assay, 20 October 2005	INF.4
5c	Validation of Screening and Testing Assays Proposed for the EDSP	INF.7
5e	SPSF for the Development of a DRP on Availability of In Vitro Receptor Assays in Fish for Screening of Endocrine Modulating Activities of Environmental Chemicals, Submitted by Japan, UK and Sweden	INF.15
6a	Results of Assay and Tests in Evaluation of the Endocrine Disrupting Activities in Fish (Medaka), Japan	INF.5
6a	Draft report of Phase 2 of the Validation of the 21-day Fish Screening Assay- Negative Substances Testing	INF.6
6a	US EPA Fish Screening Assay Discussion Paper	INF.8
7b	Summary minutes of teleconference meeting held on 6 Feb 2006 The preliminary validation assessment panel of the 'Japanese multi-laboratories validation study of a stably transfected ERalpha mediated reporter gene assay in Japan'	INF.2
7b	Draft summary minutes of teleconference meeting held on 17 March 2006 The preliminary validation assessment panel of the 'Japanese multi-laboratories validation study of a stably transfected ERα mediated reporter gene assay in Japan'	INF.16
Background Documents		
8	Please see the reports posted on the EDTA protected Website	Reports
Presentations		
6a	Completing the Validation of the OECD Fish Screen - BIAC Perspective, Willie Owens, BIAC	For information only
7a	The Use of Metabolising Systems for In Vitro Testing of Endocrine Disrupters, Walter Janssens, Scientific Institute for Public Health, Belgium	

Draft Agenda

6th Meeting of the Validation Management Group for Mammalian Effects Testing (VMG-Mammalian) of the Task Force on Endocrine Disrupters Testing and Assessment (EDTA) 17-18 January 2007, Ljubljana (Slovenia)

Wednesday 17 January		
09h00	**Agenda Item 1: Opening of the Meeting, Introduction of Participants** The meeting will be opened by Dr. Mike Wade, Chair of the VMG- mammalian. The host will welcome the participants.	
09h15	**Agenda Item 2: Approval of the Draft Agenda**	Draft agenda
09h10	**Agenda Item 3: Information from the Secretariat**	**INF 10** (status of the validation reports)
09h45	**Agenda Item 4: Approval of the report on "Additional data supporting the Test Guideline on the Uterotrophic Bioassay in Rodents"** The Secretariat has developed a specific report addressing additional issues for the validation of the Uterotrophic Bioassay. This report is made of two parts: "Additional data on the specificity of the Uterotrophic Bioassay" and "Validation of the Uterotrophic Bioassay in mice by bridging data to rats". This document was sent to the VMG-mam and the EDTA for comments by late November. Only a few comments were received leading to slight changes to the part on specificity. The VMG will be invited to approve the report.	**MD 8** (specificity - revised document) and **MD 9** (mice) **INF 7** (compiled comments) **INF 8** (Tinwell et al., 2000) **INF 9** (Markey et al., 2001)
10h30	*Coffee break*	
11h00	**Agenda Item 5: Technical issues raised during the 2 commenting rounds of the draft Test Guideline of the Uterotrophic Bioassay** The Secretariat circulated a draft version of the TG in May 2006 to the WNT for comments/approval. The Secretariat revised the draft TG on the basis of the comments received and identified technical issues to be discussed by the VMG-mam. The revised TG was sent to the WNT in July 2006 for a second commenting round. Following the second series of comments, the draft TG was revised and the antioestrogenic protocol was moved to a separate Guidance Document. The list of technical issues to be discussed by the VMG was completed. The VMG will be invited to discuss these issues and to approve the Guidance Document. With respect to the TG, which is now under discussion by the WNT, the VMG should restrict its comments to the technical issues raised during the commenting periods. **(1) Issues on measurement and influence of phytooestrogen level in the diet and bedding**	**MD 1** (draft TG revised 23 November 2006) **MD 2** (discussion paper) **MD 3** (draft GD on the antioestrogenic protocol) **INF 1** (compiled comments 1st round) **INF 2** (compiled comments 2nd round, revised 23 November) **INF 3** (comments on the discussion issues) **INF 12** (Thigpen et al., 2004) **INF 13** (Kato et al., 2004) **INF 14** (Kanno et al., 2002)

12h00	**Agenda Item 6: Technical issues raised during the 2 commenting rounds of the draft Test Guideline of the Uterotrophic Bioassay** **(2) Issues on controls and criteria for significance of positive results**	
13h00	*Lunch Break*	
14h30	**Agenda Item 7: Technical issues raised during the 2 commenting rounds of the draft Test Guideline of the Uterotrophic Bioassay** **(3) Other issues**	
15h30	**Agenda Item 8: Review of the Test Guideline of the Uterotrophic Bioassay after adoption** During the second commenting round, the US proposed that the Test Guideline be revisited after sufficient experience is gained with its use (i.e. after testing the first 50-100 chemicals). The US would welcome working with the OECD Secretariat and would be willing to take the lead in this review. Gary Timm will present the topic and the VMG will be invited to discuss on the modality of implementation of this process and how other countries could be involved.	
16h00	*Coffee Break*	
16h30	**Agenda Item 9: Approval of the Phase-3 report of the validation study of the Hershberger Bioassay – adult castrate model** The Phase-3 of the validation study of the Hershberger Bioassay was sent to the VMG-mam and the EDTA for comments by late September 2006. The Secretariat revised this document on the basis of the comments received. The VMG will be invited to approve the report, revised as appropriate.	**MD 4** (validation report) **INF 4** (compiled comments)
17h10	**Agenda Item 10: Background Review Document of the Hershberger Bioassay** The development of the Background Review Document is on the Work Programme with the U.S. as lead country. Gary Timm will present the document and will answer to questions from the VMG. This document was posted on the public website on 4 January 2007 for comments from the VMG and EDTA by 9 February 2007.	**MD 5** (BRD)
17h30	*Meeting Adjourns for the day*	

Thursday 18 January		
09h00	**Items from previous day – revised documents for approval.**	
10h30	**Agenda Item 11: Phase-3 of the validation study of the Hershberger Bioassay – weanling model** Willie Owens will present the validation work on the weanling model and first results (if available). The VMG will be invited to take note of the progress of the validation study on weanlings, comment and agree on a time schedule for the development of the validation report.	
11h00	*Coffee Break*	
11h30	**Agenda Item 12: New mammalian Level 5 test involving various life stages** Gary Timm will report progress on the work on this issue. The VMG will be invited to discuss the design of the Level 5 mammalian test and the need to form an expert group to look at this issue.	**INF 5** (SPSF) **INF 6** (Cooper et al., 2006)
12h15	**Agenda Item 13: Guidance on histopathology for the updated TG 407** At the last VMG meeting, the need of a guidance on histopathology to improve the assay power and sensitivity was discussed. It was also proposed to convene an international group of pathologists to provide information for this guidance. The VMG will be requested to confirm on the need of a guidance document on histopathology, to set the main axis of this guidance and to discuss of the constitution of the group (need for a meeting?).	
13h00	*Lunch Break*	
14h30	**Agenda Item 14: Validation white paper: Lessons Learned and Experience Offered** The EDTA task Force agreed during its last meeting to develop a paper which would present practical experience gained with validation and show how flexibility was applied. Willie Owens has developed a draft white paper on validation. Willie Owens will present the document. The VMG will be invited to give their initial thoughts on the history related in this document. The WNT will be asked whether they support the development of the document with recommendations as prepared by Willie Owens.	**MD 7** (Draft Validation White paper)

14h50	**Agenda Item 15: Endocrine disrupting chemicals screening and testing scheme project (MHLW)** Jun Kanno will present this topic and the VMG will be invited to take note on the progress made since the last VMG mammalian meeting and comment.	**INF 11**
15h10	**Agenda Item 16: Activities of the common chemical repository in the development and validation of the updated TG 407 and the Uterotrophic and Hershberger Assays.** Elard Jacob will present this topic and the VMG will be invited to take note and comment on the activities and the future of the repository.	
15h30	**Agenda Item 17: Other issues** Any new activity for the VMG? Issues to be forwarded to the EDTA Task Force Meeting; Any other issues; Date of the next meeting; Election or reelection of the chair.	
16h30	*Meeting adjourns*	

Item 15 **INF11**

Draft Report of Screening and Testing Scheme for Endocrine Disrupting Chemicals (MHLW) **updated** for OECD VMG mammalian, *January 17-18, 2007 @Slovenia*

Scheme Working Group Members

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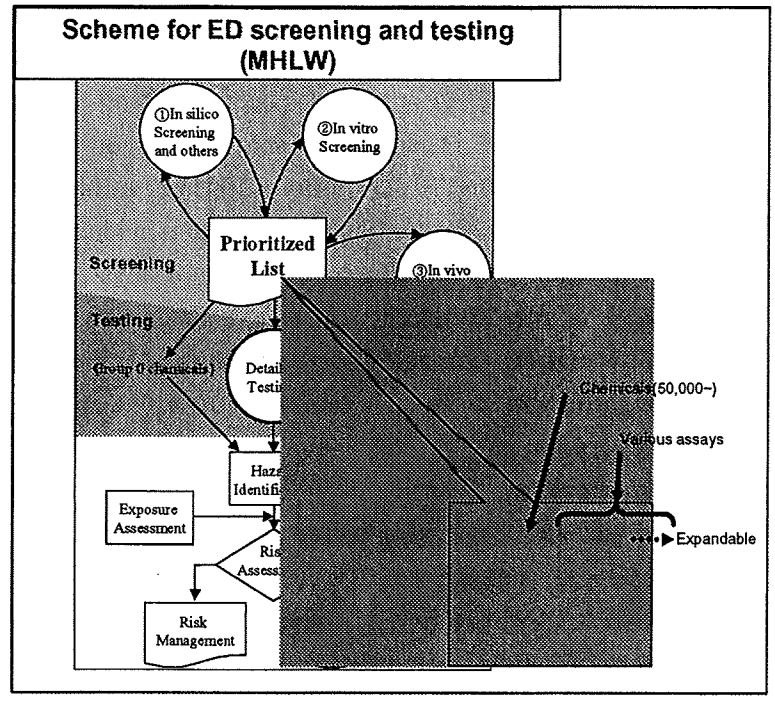
1. Introduction

The Japanese Ministry of Health Labour and Welfare has adopted a Screening and Testing Scheme for Endocrine Disrupting Chemicals. The aim of the screening is to prioritize tens of thousands of chemicals with special reference to the hormonal activities. The prioritized chemicals are then subject to the Definitive Testing for the risk assessment and following risk management.



Since 1998, Research Groups have been assembled by the support of MHLW Health Science Research grants.

For the Screening, 1) in silico, 2) in vitro and 3) in vivo methods are prepared as a battery tests to prioritize the chemicals. The Chemicals in the list is sorted by the battery test data whenever the new measurement data are added to the list (a chemical with high hormonal activity goes to the top portion of the list, whereas a chemical with low activity goes down in position). It is noted that the total number of chemicals in this list does not decrease, and only the position of the chemicals are subject to changes, so that the list will mature according to the increase in data. The category for sorting chemicals can be increased on demand and can be give different weight for the sorting. For example, the product volume can be added so that both hormonal activity and production volume of the chemicals are incorporated in the process of prioritization.

Top chemicals of the prioritized list are subject to the definitive testing, risk assessment, and risk management. The chemicals proven to be negative by the definitive testing will be kept in the hold box until any new scientific finding on possible disruption emerges.

2. Current status of the development of the Screening and Testing Methods.

Screening methods

1) In silico screening:

The receptor binding ability is predicted by the 3D-SAR using the docking model. Fully automated ER alpha and ER beta docking models are developed, and a series of virtual screenings have been conducted. About 5,000~6,000 possible binders are selected from 20,000 chemical list. Current attempt includes calculation of relative binding affinity (RBA) against 17 beta estradiol. The disadvantage of the docking model is the need of structural information of the receptor molecule and also chemicals. To reduce the possibility of false negative, the crystallographic data of the ER with antagonist is used (the binding site is wider than that of agonist binding ER). The advantage of the docking model is that it does not need teaching molecule so that chemicals of any structure can be calculated (no chemical domain is considered, except for metals). Our study showed that the docking model can be utilized with low false negative rate so that it is useful for the screening purpose.

2) In vitro screening:

i. Cell culture system:

a. Reporter gene assay using mammalian cell lines

Screening methods which monitors the hormone receptor-dependent transcription were developed and tested. Hela cell was used for ER alpha, ER alpha-antagonist, ER beta, ER beta-antagonist, and CHO cell was used for AR, AR-antagonist, TR (TR beta + RXR alpha), TR-antagonist. The number of tested compounds on each assay was shown in Table1.

Table.1 Number of compound tested in NIHS (MHLW) by TA assay

NR	Assay system		No. of compounds tested
ERalpha	stable	agonist assay	350
		antagonist assay	350
	transient	agonist assay	170
ERbeta	transient	agonist assay	170
AR	stable	agonist assay	150
		antagonist assay	150
TRbeta*	transient	agonist assay	150
		antagonist assay	150

* TRbeta / RXR co-transfected CHO cell was used for assay

b. Other assays

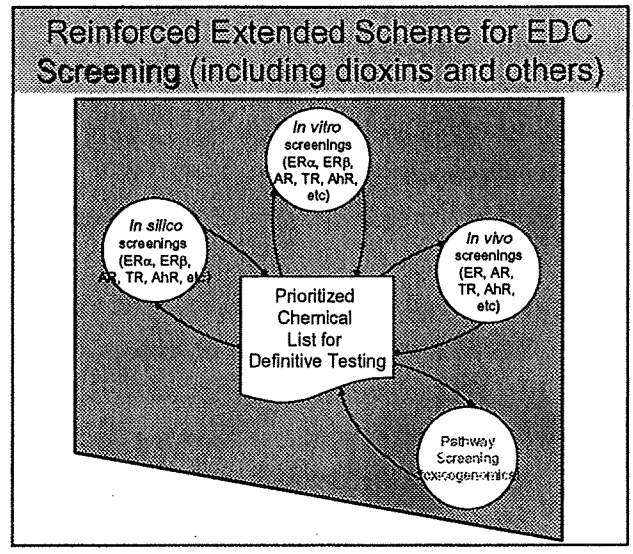
Aromatase assay using human granulosa tumor cell line (KGN cells) have been established and tested for 100 chemicals to detect aromatase activators and inhibitors. For androgenic activities, intranuclear AR dot localization assay, activin receptor assay, chemotactic assay for anti-androgens are under development.

ii. Cell-free system

Surface Plasmon Resonance system for measurement of molecular interaction between Receptor, Ligand, DNA responsive element, and co-factor are developed. Ligand dependent alteration in interaction between ER alpha and ERE sequence, ER beta and ERE sequence, ER alpha and cofactor TIF-2 LxxLL sequence, and ER beta and cofactor TIF-2 LxxLL sequence were tested for 300, 100, 300, 100 measurements respectively.

iii. Pathway screening

Additional comprehensive screening method would be the high-density cDNA microarray. We are planning to conduct a small scale in vivo microarray study to scan for the signaling pathways involved in the hormone receptor signaling system by referring to a large toxicogenomics database.



3) *In vivo* screening

The monitor in vivo activity of