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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

PROPOSAL FOR THE UTEROTROPHIC BIOASSAY VALIDATION PEER REVIEW PROCESS

4th Meeting of the Validation Management Group for Mammalian Effects Testing (VMG-Mammalian) of the Task Force on Endocrine Disrupters Testing and Assessment (EDTA) to be held at OECD Headquarters, 14th - 15th April 2003, Paris

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This document briefly describes a proposal for the peer review of the uterotrophic assay, taking into account the variety of opinions expressed on this activity by members of EDTA and WNT.

Action Required: ***The VMG-Mammalian is invited to comment on the proposal and provide further suggestions as appropriate, for consideration of the EDTA Task Force.***

INTRODUCTION

1. The process for the development, validation and adoption of a test method as OECD Test Guideline is separated into discrete conceptual phases, such as:

- Test method selection;
- Initial assessment of the relevance and reliability of the test method (test optimisation);
- Full assessment of the relevance and reliability of the test method (characterising in full the performance of the test);
- Overall evaluation of the validation study (independent peer review);
- Approval of the Draft Test Guideline by regulatory authorities of OECD member countries; and
- Adoption of the Test Guideline by the OECD Council and regulatory acceptance in member countries.

2. This process is, in practice, a continuous and sometimes overlapping flow of events. Each successive phase of the process uses and builds on results of earlier phases of the work.

3. For the uterotrophic bioassay both the initial and full assessment of the reliability and relevance of the method have been finalised and published as OECD documents [ENV/JM/TG/EDTA(2001)1 and ENV/JM/TG/EDTA(2003)1]. In addition, the work was also published in the peer reviewed scientific literature (1) (2) (3) (4) (5). From the discussions of the validation of this assay by the Task Force on EDTA and the WNT, it was clear that the interpretation of scale and rigor of the next step (the independent peer review) varied considerably among delegates. Opinions ranged from a full-size standard procedure for a peer review including the establishment and meetings of a dedicated panel of selected independent experts to the acceptance of the subsequent review by the EDTA, WNT and Joint Meeting as an adequately independent review process.

4. The 13th WNT endorsed the compromise proposal made by EDTA5 to arrange for an independent peer review test-case for the uterotrophic bioassay, including the establishment of a small independent peer review panel with a defined task and the aim to avoid face-to-face meetings, if possible. The WNT also agreed that this should not be considered as a precedent for future peer reviews: each case would be judged on its own merits.

5. This document briefly outlines the organisation of the independent peer review of the validation of the uterotrophic bioassay. The document outlines a work plan, criteria to be used, and actions to conduct the peer review for this bioassay

THE INDEPENDENT PEER REVIEW AND EVALUATION OF THE UTEROTROPHIC BIOASSAY VALIDATION.

6. This independent evaluation, referred to as a peer review, is conducted to ensure that the various activities were technically adequate, competently performed, properly documented, and satisfy established quality requirements. It is intended to identify any technical problems or unresolved issues so that, if any, these may be resolved prior to the adoption of the method as Test Guideline. The peer review will be conducted by experts, technically qualified in the field, who are independent of the experts who were involved in the validation work (absence of previous substantive involvement in the program) and have no other conflicts of interest (biases or interests that might substantially influence their judgment in either a positive or negative way).

7. The Secretariat will request the EDTA and WNT to nominate independent experts to the *ad hoc* Peer Review Panel (PRP). In order to facilitate the selection of most appropriate peer reviewers, the Secretariat will provide member country delegates with guidance on what may be considered as "independent" in the context of the review of the uterotrophic bioassay. A PRP of six to ten members is considered more effective and efficient than a much larger or smaller group.

8. The peer review will be performed after the report of Phase 2 of the validation study [ENV/JM/TG/EDTA(2003)1] has been approved by the VMG-mammalian, amended as appropriate. This report together with the already approved report of Phase 1 [ENV/JM/TG/EDTA(2002)1] form the foundation of material to be submitted for peer review. In addition, the following documents will be submitted to the peer reviewers:

- EHS Monograph No.38 in the Series on Testing and Assessment: Detailed Background Review of the Uterotrophic Bioassay;
- The protocols used in the Uterotrophic Bioassay Validation Program;
- The published or accepted manuscripts related to the validation of the Uterotrophic Bioassay, i.e. (1) (2) (3) (4) (5).

9. In addition, any particular set of individual data as well as statistical reports not already included in the Phase 1 and 2 reports will be made available to reviewers upon request only. The reason for this restriction is that the volume of the total package of individual data does not allow regular electronic transmission or even paper mailing.

10. The peer reviewers will also be provided with general background information on the Test Guidelines Programme and internationally accepted validation approaches as described in the following documents:

- EHS Monograph No.1 in the Series on Testing and Assessment: Guidance Document for the Development of OECD Guidelines for the Testing of Chemicals;
- (Draft) EHS Monograph No 34 in the Series on Testing and Assessment: Guidance Document on the Development, Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment;

11. The process of conducting this peer review is likely to include the following steps:

- Nomination of independent experts and establishment of the Peer Review Panel (PRP);
- Teleconference of the PRP to explain the goals for the peer review and the charge to the PRP as explained in the "Guidance and Charge to the Peer Review Panel" (see Annex 1);
- It is anticipated that the interactions among the PRP during the review process will be by e-mail and other written correspondence, and followed by one or more telephone conference calls. This will involve the input of each member to items in the charge so that the identification of possible issues be rapidly identified. Where necessary, clarification or additional details at this stage may be necessary. These can be requested by the PRP from the Secretariat, who will contact the appropriate parties involved in the validation program and will be provided to the PRP, if available.
- After completion of the review, each member of the PRP will draft his/her review report following the guidance and questions asked (see Annex 1).
- The Secretariat will compile and collate all individual review reports and provide a comparative analysis of the peer review, together with the compilation, to all members of the PRP.

- A teleconference will be arranged to settle, if possible, any discrepancies between individual reviews
- In case of persistent differences of opinion, a face-to-face meeting of the PRP may be needed, which may or may not include parties involved in the Uterotrophic validation program to respond to PRP questions.
- The final report of the peer review, that will include all individual reports, will have to be signed by all members of the PRP before it can be submitted to the EDTA Task Force with a copy to the VMG-mammalian and the WNT.

CRITERIA FOR THE SELECTION OF PEER REVIEWERS

12. The experts who will serve as peer reviewers of the uterotrophic bioassay validation project should be technically competent, credible, and able to perform their assessment and writing tasks. In addition the peer reviewers should not have financial or other conflicts of interest with the bioassay ort he outcome of the peer review. The following specific selection criteria are proposed to cover these general conditions:

A. The experts selected for the PRP should have demonstrated expertise in one or more of the relevant scientific disciplines necessary to review the Uterotrophic Bioassay Validation project. The fields of expertise and subject matters include, but would not be limited to:

- The endocrinology of steroid hormones and particularly estrogens,
- The molecular and cellular biology of estrogen action,
- The reproductive toxicology of estrogens, particularly the elicitation of adverse effects in regulatory developmental and reproductive protocols,
- Biostatistics, particularly the analysis of multi-laboratory data sets,
- Inter- and intra-laboratory comparison and validation studies of methods and substances,
- Animal welfare issues and the associated three R's, i.e., replacement, reduction, and
- Regulatory application of toxicology, including hazard and risk assessment.

Note: Together the PRP should cover each of the identified disciplines. Therefore, it may be useful to select members who are technical experts in more than one of the listed fields.

B. It is essential that peer reviewers be independent and have no conflict of interest, such as:

- Previous substantial involvement with the Uterotrophic Bioassay Validation Project,
- Material or philosophical interest in the outcome of the review, either positive or negative.

Note: It is generally understood that a conflict of interest also means that the partner of the expert ("partner" includes a spouse or other persons with whom the expert has a close personal relationship), or the administrative unit with which the expert has an employment relationship, has/have a financial or other interest that could unduly influence the expert's position on the peer review outcome.

13. As the explicit goal of the validation of the Uterotrophic Bioassay is the adoption of the method as a new OECD Test Guideline, it would be helpful if at least some of the reviewers have knowledge of the OECD Test Guidelines Programme and are familiar with the process of Test Guideline development and adoption.

14. In order to protect the integrity and independence of the PRP, members should avoid discussing substantive issues pertaining to the peer review with outside parties during the conduct of the peer review. In the event that parties contact PRP members with suggestions or other issues related to the validation project, PRP members should preferably refer those persons to the Secretariat. During the review process, the PRP members should not release written comments to outside parties.

CHARGE TO THE PRP FOR THE UTEROTROPHIC BIOASSAY

15. The charge to the peer reviewers will pose searching and insightful questions that elicit input on the strengths and limitations of the critical aspects of both the toxicity test and the validation study. The charge should be complete, objective, and credible. A proposal for the charge for the Uterotrophic Bioassay PRP is provided in Annex 1.

REFERENCES

(1) Owens JW, Ashby J. (2002). Critical Review and Evaluation of the Uterotrophic Bioassay for the Identification of Possible Estrogen Agonists and Antagonists: In Support of the Validation of the OECD Uterotrophic Protocols for the Laboratory Rodent. Crit. Rev. Toxicol. 32: In press.

(2) Kanno, J, Onyon L, Haseman J, Fenner-Crisp P, Ashby J, Owens W. (2001). The OECD program to validate the rat uterotrophic bioassay to screen compounds for in vivo estrogenic responses: Phase 1. Environ Health Perspect. 109:785-94.

(3) Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. (2003a). The OECD program to validate the rat uterotrophic bioassay: Phase Two - Dose Response Studies. Environ. Health Persp. (in press).

(4) Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. (2003b). The OECD program to validate the rat uterotrophic bioassay: Phase Two – Coded Single Dose Studies. Environ. Health Persp. (in press).

(5) Owens W, Ashby J, Odum J, Onyon L. (2003). The OECD program to validate the rat uterotrophic bioassay: Phase Two – Dietary phytoestrogen analyses. Environ. Health Persp. (accepted for publication).

ANNEX 1

DRAFT GUIDANCE AND CHARGE TO THE PEER REVIEW PANEL

INSTRUCTIONS FOR PEER REVIEW PANEL (PRP) MEMBERS

1. The Peer Review Panel (PRP) is charged with developing a consensus on the usefulness and limitations of the Rodent Uterotrophic Bioassay as a robust, reliable and relevant *in vivo* method for the detection of substances that have the potential to act like and, consequently, interfere with endogenous female sex hormones. Thus, the Rodent Uterotrophic Bioassay is expected to identify chemical substances that act like oestrogen agonists or antagonists.

2. In reaching its determination, the PRP is asked to evaluate all of the available information in the Submission Package. The PRP should act in accordance with the published OECD criteria for validation and acceptance of toxicological test methods (1) (2).

3. Each member of the PRP is charged with preparing a written report that addresses each of the questions outlined in this Guidance and provides an overall conclusion.

4. The Submission Package comprises:

- EHS Monograph No.38 in the Series on Testing and Assessment: Detailed Background Review of the Uterotrophic Bioassay;
- ENV/JM/TG/EDTA(2002)1: OECD Report of the Initial Work Towards the Validation of the Rodent Uterotrophic Assay: Phase One;
- ENV/JM/TG/EDTA(2003)1/REV1: OECD Report of the Validation of the Rat Uterotrophic Bioassay: Phase 2:. Testing of Potent and Weak Oestrogen Agonists by Multiple Laboratories;
- The protocols used in the Uterotrophic Bioassay Validation Program;
- The published or accepted manuscripts related to the validation of the Uterotrophic Bioassay, i.e.
    (1) Owens JW, Ashby J. (2002). Critical Review and Evaluation of the Uterotrophic Bioassay for the Identification of Possible Estrogen Agonists and Antagonists: In Support of the Validation of the OECD Uterotrophic Protocols for the Laboratory Rodent. Crit. Rev. Toxicol. 32: In press.
    (2) Kanno, J, Onyon L, Haseman J, Fenner-Crisp P, Ashby J, Owens W. (2001). The OECD program to validate the rat uterotrophic bioassay to screen compounds for in vivo estrogenic responses: Phase 1. Environ Health Perspect. 109:785-94.
    (3) Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. (2003a). The OECD program to validate the rat uterotrophic bioassay: Phase Two - Dose Response Studies. Environ. Health Persp. (in press).
    (4) Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. (2003b). The OECD program to validate the rat uterotrophic bioassay: Phase Two – Coded Single Dose Studies. Environ. Health Persp. (in press).
    (5) Owens W, Ashby J, Odum J, Onyon L. (2003). The OECD program to validate the rat uterotrophic bioassay: Phase Two – Dietary phytoestrogen analyses. Environ. Health Persp. (accepted for publication).
- EHS Monograph No.1 in the Series on Testing and Assessment: Guidance Document for the Development of OECD Guidelines for the Testing of Chemicals;
- (Draft) EHS Monograph No 34 in the Series on Testing and Assessment: Guidance Document on the Development, Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment;

- Upon request: any individual data generated as part of the Uterotrophic Bioassay Validation Project;
- Upon request: any detail of the statistical reports associated with the Uterotrophic Bioassay Validation Program.

5. In conducting the peer review, the primary focus of the PRP is to evaluate the proposed Uterotrophic Bioassay protocols and supporting submission materials. Based on this information, the PRP is asked to focus on the following questions:

> **Has the Uterotrophic Bioassay been sufficiently evaluated and has its performance been satisfactorily characterized to support its proposed use for screening the potential of chemical substances to act as oestrogen agonists and antagonists *in vivo?***

POINTS FOR EVALUATION

The Test System

6. Is the choice of the rodent uterus, specifically in this case the rat uterus:

a. Inherently biologically relevant for the detection of oestrogen agonists and antagonists in vivo?
b. Mechanistically adequate and sensitive for the detection of oestrogen agonists and antagonists in vivo?
c. Toxicological an appropriate choice for the detection of oestrogen agonists and antagonists *in vivo*?
d. Consistent with the use of animals to obtain *in vivo* hazard information for human hazard assessment?

Test Method and Protocol Description

7. Are the test method and protocols described in sufficient detail in the Submission Package, including the scientific and mechanistic basis of the test, endpoints, protocol parameters, and acceptable variations among the protocols?

8. Are the protocols used to generate the supporting submission data complete and adequate in detail for a laboratory to conduct the study, including:

a. Description of the material and equipment needed to conduct the test.
b. Description of what is measured and how the data are used to identify positive and negative results?
c. Are there appropriate provisions for the use of reference control chemicals?

9. What are the strengths and/or limitations of the Uterotrophic Bioassay and are they adequately described?

10. Are there editorial/technical corrections necessary for the proposed protocol?

Test Method Data Quality and Sufficiency

11. Is there evidence that the data generated during the validation studies are of sufficient quality, including adherence to the protocol and a clear description and justification of any deviations? This might include evidence that the data were generated in compliance with Good Laboratory Practices.

12. Are the data provided in sufficient detail to evaluate the results and performance of the Uterotrophic Bioassay for its proposed use?

Test Method Performance

13. Were the characteristics of the test substances selected adequate to demonstrate the performance of the Uterotrophic Bioassay for its intended use as an *in vivo* screen for oestrogen agonist and antagonist activity? Does the selection adequately represent the types of substances for which the test method is proposed to be used? Is it then appropriate to generalise the performance of the method for all test substances or are there important limitations on the applicability of the Uterotrophic Bioassay to certain test substances?

14. Was the use of the test substances in dose response experiments adequate to demonstrate the toxicological performance of the Uterotrophic Bioassay for its intended use as an *in vivo* test for oestrogen agonist and antagonist activity?

15. Were the statistical/analytical methods used adequate to evaluate the performance of the Uterotrophic Bioassay?

16. Does the Submission Package adequately support the utility of the method for regulatory use in hazard assessment of chemical substances that may have the potential to act as oestrogen agonists and/or antagonists?

Determination of Test Method Reliability (Repeatability/Reproducibility)

17. Have the intra- and inter-laboratory reproducibility of the Uterotrophic Bioassay been adequately evaluated

a. taking into account the need to balance the use of resources and the generation of data, comment on the adequacy of the evaluation of intra- and interlaboratory reproducibility of the test method?
b. taking into account the objective of providing a Test Guideline that can be used widely and internationally allowing the necessary flexibility in the selection of strain, diet, bedding, vehicle, and other conditions?

18. Was the reproducibility of the test method adequately evaluated using coded (blinded samples)?

19. Considering the variability inherent in all chemical and biological test methods, are the results obtained with the Uterotrophic Bioassay sufficiently repeatable and reproducible?

Other Considerations

20. Considering the need to employ the Uterotrophic Bioassay internationally as a test, can the test method be readily transferred among properly equipped and staffed laboratories? Specifically comment on the following:

a. Is the Uterotrophic Bioassay relatively insensitive to minor changes in protocol?
b. Are there any patent or proprietary issues that will inhibit the use of the Uterotrophic Bioassay?
c. Are the apparent level of training and expertise required to conduct the test reasonable for its wide use as a test?
d. Are the necessary equipment and supplies relatively easy to obtain?
e. Is the method cost-effective, relative to the cost of conducting other *in vivo* assays?
f. Is the time needed to conduct the test reasonable?
g. Are there complications or limitations that have not been addressed by the protocols?
h. Is there any other information that should have been be added to the Submission Package, published or un-published?
i. Has there been adequate consideration and appropriate incorporation of animal use, refinement, and reduction in the protocol, e.g., the group size of six animals? Is the Uterotrophic Bioassay likely to cause unnecessary use of animals in the testing of positive compounds?

Summary Conclusions

21. Based on the information provided in the Submission Package:

a. Does this method adequately identify the potential for test substances to act *in vivo* as possible oestrogen agonists and antagonists?
b. Are there currently available methods that can provide equivalent or better *in vivo* predictions of oestrogen agonists and antagonist (reliability), with equivalent or better relevance, with equivalent or lower costs, and with equivalent or lower use animals?
c. Discuss conditions/limitations/restrictions that may affect the intended use of Uterotrophic Bioassay, and that are justified based upon the current presence or lack of scientific evidence.

REFERENCES

(1) OECD (1996). Final Report of the OECD Workshop on the Harmonisation of Validation and Acceptance Criteria for Alternative Toxicological Test Methods (Solna Report, 1996) as presented to the Seventh Meeting of the National Co-ordinators of the Test Guidelines Programme, 18th-19th September 1996. ENV/MC/CHEM/TG(96)9. Paris: OECD, 1996.

(2) OECD (2002). Final Report of the OECD Conference on the Validation and Regulatory Acceptance of New and Updated Methods in Hazard Assessment (Stockholm Report). ENV/JM/TG/M(2002)2. Paris: OECD, 2002.