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SUMMARY REPORT OF THE UTEROTROPHIC BIOASSAY PEER REVIEW PANEL, INCLUDING AGREEMENT OF THE WORKING GROUP OF NATIONAL COORDINATORS OF THE TEST GUIDELINES PROGRAMME ON THE FOLLOW-UP OF THIS REPORT

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or contact:

OECD Environment Directorate,
Environment, Health and Safety Division
2 rue André-Pascal
75775 Paris Cedex 16
France

Fax: (33-1) 44 30 61 80
E-mail: ehscont@oecd.org

FOREWORD

This document presents the summary report of the Uterotrophic Bioassay Peer Review Panel (PRP), preceded by the agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the follow up of the PRP report.

The project to develop a Test Guideline for the Uterotrophic Bioassay was included in the work plan of the Test Guidelines Programme ten years ago. The rodent Uterotrophic Bioassay then underwent an extensive validation program including the compilation of a detailed background document and the conduct of extensive intra-and inter-laboratory studies to show the reliability and reproducibility of the bioassay with potent reference oestrogens, weak oestrogen receptor agonists, a strong oestrogen receptor antagonist and a negative reference chemical. The validation was reviewed by a peer review panel.

Considering that members of the peer review panel were not informed of the agreement to declassify the results of their discussions, the Working Group of the National Coordinators of the Test Guidelines Programme agreed that the declassification should be proposed for a summary of the report only. At its 18th meeting, which was held in Switzerland on 16-18 May 2006, the WNT requested that its agreement on the follow up of PRP report be attached to the summary PRP report.

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

Joint Meeting Statement on the Context of Declassification of the Summary Report on the Peer Review of the Uterotrophic Bioassay

This report reflects the peer review of a study done to validate a uterotrophic protocol for testing of endocrine disrupting activity. The protocol was developed by experts of an OECD Validation Management Group established by the OECD Endocrine Disruption Testing and Assessment Task Force.

The validation of this protocol included the testing of a number of substances by different laboratories for the evaluation of the reliability, reproducibility and relevance of the protocol. The peer review panel was asked to give its views on the validation report. The peer review panel did not reach a consensus but were able to agree a summary report.

Agreement of the Working Group of the National Coordinators of the Test Guidelines Programme on the Follow up of the Peer Review Panel Report

The Working Group of the National Coordinators of the Test Guidelines Programme (WNT) considered the outcomes from the discussion by the Validation Management Group on Mammalian Effects Testing (VMG-mammalian) and of the Endocrine Disrupters Testing and Assessment Task Force (EDTA TF) of the PRP report. It supported the development of a retrospective study on additional data to address the specificity of the assay; this was considered as the most important follow-up of the peer review report. It also noted that experience with the Test Guideline will provide further information, e.g. on substances with enhanced activity due to metabolism and on the specificity and sensitivity of the test for anti-oestrogenic activity. Moreover, the WNT welcomed the development of a report on bridging data to support the use of mice in the assay.

Considering the above, and also considering the urgent regulatory need for Guidelines for the testing of endocrine disrupters, the WNT agreed to proceed to the finalization of a draft OECD Test Guideline for screening chemicals with oestrogenic activity and for providing supporting evidence in relation to anti-oestrogenic activity.

Summary Report of the Peer Review Panel for the Uterotrophic Bioassay

1. The peer review panel (PRP) was constituted in September 2003, to provide a review of the validation process for the Uterotrophic Bioassay, to evaluate the data collected, and to answer specific questions posed to the Panel in the charge provided by the sponsoring organization, the Organization for Economic Cooperation and Development (the OECD). The Panel held several teleconferences, and each Panel member submitted written answers to the charge questions to the Secretariat prior to each teleconference. This report presents the combined PRP responses to each of these charge questions.

2. The Peer Review Panel was requested to report their views on the validation process for the Uterotrophic Assay to the Validation Management Group-mammalian (VMG-mammalian) responsible for overseeing the validation process, and then to the Endocrine Disruptor Testing and Assessment Taskforce (EDTA), and the Working Group of the National Coordinators of the Test Guidelines Programme (WNT). Taking this peer review report into consideration, the EDTA and WNT will recommend any further activities on this OECD project, including the process for the development of a Test Guideline.

3. Regarding the overall validation exercise, the final conclusions and the views of the PRP are divided into broad groups and there were considerable differences expressed regarding the various components of the project. The PRP was unable to reach consensus on the issue of the validation status of the uterotrophic assay, and the differences in opinion between PRP members were significant. Some members considered the Uterotrophic Bioassay to be validated for the intended purpose of the assay, other members considered that further data, including on negative substances, was necessary to reach a decision on the validation status of the assay, whilst other members considered the efforts to date were not sufficient to validate the test method but to only be sufficient as a pre-validation study. The difficulty in reconciling such differences should be considered when reading the individual responses to the charge questions that follow.

Background

4. The National Coordinators (WNT) agreed to establish a special activity to address the issue of endocrine disruption and develop new Test Guidelines as appropriate. The responsible body was the Task Force for Endocrine Disruptor Testing and Assessment (EDTA). The EDTA agreed to initially pursue efforts to develop and validate Test Guidelines for the uterotrophic assay and the Hershberger assay, and to evaluate enhancements to the current Test Guideline 407. A single Validation Management Group (VMG) was established to manage these projects. Subsequently, the EDTA has begun activities concerning ecotoxicity testing and *in vitro* or non-animal testing also related to the endocrine disruption issue. As a result, the original VMG is now divided into the VMG for mammalian effects assessment (VMG-mammalian), the VMG for ecotoxicity testing (VMG-eco) and the VMG for non-animal testing (VMG-NA) to manage the diverse activities and work loads.

5. The VMG-mammalian managed the uterotrophic activity, the first step of which was to evaluate and propose the standardization of protocols to address the use of both the immature female rat and ovariectomized female rat, as well as different routes of administration. The VMG-mammalian designed and conducted an initial phase (Phase-1) with the potent oestrogen compound, ethinyl oestradiol, to demonstrate the transferability and reproducibility of the protocols with studies conducted in some 20 laboratories. The VMG-mammalian then initiated Phase-2 with weak oestrogen agonists both in dose response studies and in studies where these same chemicals were coded. These extensive data sets were analyzed by independent statisticians and provided to the PRP, and many of the data have been published in the scientific literature.

6. The charge of the PRP was to report on the biological and toxicological relevance of the assay, the adequacy of the protocol, the extensive set of data generated in Phases-1 and -2, and the analyses of that data. The PRP was also asked to determine whether the assay was 'validated' to meet the requirements for development of a test guideline based on this test method.

7. To facilitate this report, a series of more than 30 questions were posed to the PRP and the responses to the questions (attached) reflect the individual views of the panel. The more detailed discussions on these issues are provided in the individual reports of the teleconferences. A summary of the views of the Panel members is provided below. Where agreement between the Panel members was reached, this has been indicated. Where agreement was not reached, the text below provides a short summary of the differing views of the panel members on specific issues.

Summary of Panel Responses to Issues

8. The Peer Review Panel agreed that the biology of the test system was appropriate, as the rat uterus is a biologically relevant test system for detecting oestrogen-like biological effects *in vivo*. A majority of the Panel members agreed that the assay was adequate to test effects of oestrogen agonists for the purposes of the assay (which is designed to be used as an *in vivo* screening assay), while recognizing that there are a number of details that need to be clarified in a finalised test method. For example, instructions are necessary for the specifics on dose-setting (including what constitutes a maximum tolerated dose to avoid animal pain and suffering, and if there is to be a limit dose as in most test guidelines); the appropriate statistics for the test method; allowable limits for phytoestrogens in feed; criteria for accepting data as high quality; and, importantly, criteria for determining if a compound is positive or negative. Notwithstanding these issues, this subgroup of PRP members supported the usefulness of the test and agreed that validation is achievable for the purpose of the test, possibly with the addition of some further information on the performance of the assay with other substances.

9. Some members of the PRP expressed the need for additional information on negative compounds, due to the need for negative compounds, in addition to positives, to fully assess the assay in terms of specificity and sensitivity. Some of the PRP members indicated that such information could be obtained in a retrospective manner, from existing studies, and this information would allow further decisions to be made regarding the usefulness of the assay to screen for oestrogen antagonists.

10. Another group of the PRP members believed that the shortcomings in the current validation effort are significant and suggested that the current test should be considered to be in a 'pre-validation stage' only. That is, in the opinion of these PRP members, a validation process is yet to properly begin. This group emphasized the need to include, and to test the response of the Uterotrophic Bioassay with, additional negative substances, and recommended the need for additional metabolically-enhanced positive substances be included where possible. One view of some PRP members and observers of this group is that the testing of negatives was insufficient and would prevent the validation effort from moving from this 'pre-validation' phase.

11. Some PRP members indicated that the assay should meet the validation requirements of regional or national bodies such as ECVAM and ICCVAM, which specialise in validation of *in vitro* and other alternative methods. Some members also stated that the assay, while detecting an increase in uterine weight, did not necessarily confirm that such an effect was attributable to oestrogen agonists, and that other tests (such as receptor binding) should be utilised in conjunction with the uterotrophic assay. This would allow the assay to be used as a screen for detection of oestrogenic effects. On this issue, some panel members stressed that the validity of any bioassay must be assessed within the context of its purpose and the conditions of its intended use. Thus, if the purpose of the uterotrophic assay is to flag a chemical for further evaluation, and will not be used to label the chemical as having a certain type of activity or

mechanism of action, the inability of the assay to distinguish between substances that increase uterine weight via oestrogenic agonism versus other, as yet undefined, pathways is not a basis for rejecting the validated status of the assay.

12. Several PRP members stated that the validation of *in vitro* assays should be a priority for the OECD, as these alternatives promise lower costs and would reduce the number of animals used in the Uterotrophic Bioassay. Other PRP members identified the regulatory needs of OECD member countries for this test method as a rationale for completing the validation of the assay prior to other validation activities being undertaken.

Recommendations

13. The Peer Review Panel agrees that this report provides a summary of their views on the status of the validation of the uterotrophic assay, as detailed in the responses to the questions posed to the Panel members in the form, and based on the information on the validation exercise provided to the Peer Review Panel.

14. The report of the Peer Review Panel, along with the information developed on the validation of the uterotrophic bioassay, should form the basis for decisions on whether the validation exercise meets the OECD principles for validation for development of this test method into an OECD Test Guideline. In this consideration, the OECD should note the various views of members of the PRP. The PRP recommends that the OECD consider the PRP report, along with the validation information, to decide on additional work needed to finalize the validation exercise for the purposes of developing an OECD Test Guideline.