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Number 81**

**SUMMARY REPORT OF THE VALIDATION PEER REVIEW FOR THE UPDATED  
TEST GUIDELINE 407, AND AGREEMENT OF THE WORKING GROUP OF THE  
NATIONAL COORDINATORS OF THE TEST GUIDELINES PROGRAMME ON THE  
FOLLOW-UP OF THIS REPORT**

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## FOREWORD

This document presents the peer review summary report for the validation of the Updated Test Guideline 407, preceded by the agreement of the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) on the follow up of the PRP report.

The peer review was managed by two independent consultants. The National Coordinators proposed peer reviewers but the final composition of the independent peer review panel was decided by the consultants. The panel included 6 peer reviewers who had been proposed by Germany, Japan, the European Commission and the United States, and one observer involved in the validation. The role of the observer was to respond to technical questions related to the validation. The peer reviewers were requested to send declaration of interests to the consultants. The documents submitted to the peer review panel were posted on the OECD public website and consisted of the validation report and its annexes, the preliminary draft updated Test Guideline 407, the current Test Guideline 407, a reference to a peer reviewed publication and a Secretariat document explaining how the 8 OECD validation criteria and principles had been addressed.

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the Organisation or of the governments of its member countries.

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**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 peer review panel summary report of the validation of the updated TG 407 was submitted for information at the 19<sup>th</sup> meeting of the Working Group of National Coordinators of the Test Guidelines Programme (WNT) in March 2007. In accordance with the recommendations of the peer review panel, the WNT supported the development of a Guidance Document on histopathology to improve the performance of the Test Guideline. This document should also provide guidance on data interpretation. Furthermore, the WNT noted that compounds with weak (anti)estrogenic or (anti)androgenic properties would, in most cases, not be identified through the updated Test Guideline and agreed that this limitation should be clarified. In particular, it should be specified in the Test Guideline that it can not be considered as a screening assay for endocrine activity. The WNT requested that the Validation Management Group for Mammalian Testing addresses technical issues, raised by the Peer Review Panel or by the WNT, and proposes solutions to solve them.

Considering the above and also considering the benefit of the updated Test Guideline 407 for the detection of some endocrine active chemicals, the WNT agreed to proceed to the development and finalisation of the draft updated OECD Test Guideline 407.

## Summary Report of the Peer Review Panel for the Updated TG 407

The Peer Review Panel (PRP) was constituted in September 2006, to provide a review of the validation process for the draft updated Test Guideline (TG) 407, and to consider the specific criteria posed to the Panel by the sponsoring organization (set-out in Guidance Document GD 34), the Organisation for Economic Cooperation and Development (OECD). The Panel held several teleconferences, and each Panel member submitted written responses to the criteria posed. This report presents the combined PRP responses to the validation process.

The Peer Review Panel was requested to report their views on the validation process for the draft updated TG 407 to the Validation Management Group-mammalian testing (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). On the basis of this report the EDTA and WNT will recommend any further activities on this OECD project, including the process for completion of the development of a Test Guideline.

This report reflects the consensus view of the PRP. However there were significant differences expressed regarding the various criteria posed. These differences are described in the report and should be considered by the EDTA and WNT in making further decisions for the development of the updated TG 407.

### Background

As part of its programme of work the WNT agreed to establish a special activity to address the issue of endocrine disruption and develop new Test Guidelines as appropriate. This work was assigned to 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. The Validation Management Group – mammalian testing (VMG-mammalian) was established to manage these projects. Subsequently, the EDTA began activities concerning ecotoxicity testing and *in vitro* or non-animal testing also related to the endocrine disruption issue. As a result, further VMGs, namely the VMG for ecotoxicity testing (VMG-eco) and the VMG for non-animal testing (VMG-NA) were established to manage the diverse activities and work loads.

### Introductory Remarks

In the course of discussions on the validation exercise and the scope of the PRP's remit it was strongly pointed out by a member of the PRP that its task was to peer review the validation exercise and not the test guideline (TG). However, the PRP as a whole agreed that it would be impossible to carry out its task without considering and commenting on some elements of the TG.

Several members of the PRP believed that the long period from the initial development of the updated TG 407 to the validation exercise and the PRP activity means that the TG may not now be up-to-date. Currently there is no real alternative OECD or other protocol for investigating general toxicity and endocrine mediated effects in young adult animals. However, there is considerable activity globally in the field of in-vitro testing, quantitative and qualitative structure activity relationships, and extended one generation (Level 5) *in vivo* tests which is likely to mean that the role of updated TG 407 may change. Some members thought that, in its present form, the usefulness and applicability of updated TG 407 is

likely to be quite short as it will be made obsolete by new approaches and TGs although this will depend in part on regulatory needs.

### **Summary of the Peer Review of the Validation Exercise**

The updated TG 407 would be a useful update of the existing TG to be used for the detection of some endocrine active chemicals and, as such, a helpful addition to the portfolio of test guidelines validated by the OECD. However, the PRP believe that its benefits would be enhanced by clarifying the limitations of the updated TG (TG 407 is not performed in a life-stage that is most sensitive to endocrine disruption) and by providing additional guidance on the interpretation of test results. The PRP were clear that these two provisos did not override the benefits that would arise from the updated TG 407. The results from application of an updated TG 407, and the additional end-points covered, would be useful in many cases as such testing was required in several regulatory systems. In addition, the negative implications for animal welfare of further work on the updated TG 407 outweighed the likely benefits of such work as long as the limitations were made clear. In particular, regulators should be advised that decisions on hazard and risk management measures regarding endocrine mode of action should not be based purely on the outcome of the application of this TG; a weight-of-evidence approach should be used including results from application of this TG.

The principle limitations identified by the PRP were that the TG identified only strong or moderate endocrine active substances (EAS); it was clear that the TG could not be depended upon to identify weakly acting substances. It was also unclear from the validation exercise the expected rate of 'false-positives' and 'false-negatives' (the issue of a 'false-positive' is problematic here as changes in a given organ e.g. the prostate, could be due to endocrine activity, metabolic modulation resulting in decreased circulating testosterone levels or brought about by an unknown mechanism). Evaluation of potential endocrine activity should not therefore be based on the results of this TG alone. As weak EAS would in most cases not be identified through this TG it could not be described as a screening test able to detect ambient levels of EAS\*. It was also considered by the PRP to be too expensive and wasteful of animals for a screening test. Whilst it is a 'stand-alone' TG, the results may point to the need for further examination and/or possibly testing.

[\*the Observer of the PRP thought that this sentence was potentially misleading. His view was that detection of ambient levels (of EAS) is not a necessary characteristic of screening assays (for EAS). *In vivo* and *in vitro* screening methods can fail when it comes to the detection of endocrine activity at ambient substance levels especially in food (the most relevant route of human exposure). Besides the inherent properties of a given assay, testing of increasing doses/concentrations up to MTD/cytotoxicity/limit of solubility is used to achieve sensitivity of the assay. The Observer proposed that the sentence should read: "As weak EAS would in most cases not be identified through this TG, it could not be described as a screening test."]

### **Recommendations**

The Peer Review Panel agrees that this report provides a summary of their views on the status of the validation of the updated TG 407, as detailed in the responses to the criteria (from GD 34) posed to the Panel members and based on the information on the validation exercise provided to the Peer Review Panel.

The report of the Peer Review Panel, along with the information developed on the validation of the updated TG 407, 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 any additional work needed to finalise the validation exercise for the purposes of developing an OECD Test Guideline.

### **Summary of Peer Reviewers Comments against Validation Criteria**

This section of the report summarises the responses of the Peer Review Panel (PRP) against the validation criteria set-out in Guidance Document (GD) 34. In the interests of completeness it also includes a summary of the status of the validation **before** the start of the peer review process. This was prepared by the OECD Secretariat to present the current status of the validation of the updated TG 407, within the context of the validation criteria, and was part of the package of documents made available to the PRP. It outlines how each of the 8 criteria have been addressed during the OECD exercise and how the available information supports the validity of this method. This element of this report has not been agreed by the PRP itself. It is intended to help the reader understand the context of the PRP comments without reference to several other documents. It should be stressed that the OECD Secretariat had no involvement in the deliberations of the PRP. References to different documents of the peer review package are identified as presented below and followed by the relevant paragraph number:

- Validation report: VR
- Draft test Guideline: DTG

Some members of the PRP wished to record detailed comments that either arose from consideration of the validation criteria or from consideration of the draft updated TG itself. These unattributed comments are included at Annex 1.

**Criterion 1: The rationale for the test method should be available.**

This should include a clear statement of the scientific basis, regulatory purpose and need for the test.

**Current Status of the Validation (OECD Secretariat)**

**Scientific basis**

Ambient levels of natural and industrial chemicals may interact with the endocrine system and as a consequence possibly elicit reproductive, developmental, and other adverse effects in humans and wildlife. The supporting evidence for these concerns has been expressed in a number of expert reviews. This leads then to the need to ensure that endpoints sensitive to these mechanisms are evaluated for the usefulness and that appropriate endpoints are incorporated into toxicity tests such as the TG 407 (VR, 3).

**Regulatory purpose and need for the test**

The regulatory need for this assay stems from the report that existing Test Guidelines were generally insufficient to identify certain endocrine mechanisms (oestrogen, androgen, and thyroid) and might not be adequate to fully characterize the hazards of these mechanisms (VR, 4).

The OECD initiative to develop and validate *in vitro* and *in vivo* assays for the detection of chemicals that may interfere with the endocrine response was taken following the recommendations of a number of national, regional and international workshops and following a detailed OECD review of the status of existing test and research methods.

The proposed updates to the TG 407 are intended to potentially address both (anti)estrogens and (anti)androgens as well as thyroid toxicants. In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses is central to hazard identification and characterization. The 28-day repeat dose study (TG 407) provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The current version of TG 407 provides information on a wide range of toxic effects and indicates target organs, but is not designed to identify particular mechanisms or modes of action. The study also serves as a dose range finder for follow-up studies and provides an estimate of a no-observed-adverse-effect level (NOAEL) of exposure for certain toxic effects, and potentially a maximum tolerated dose. This information can be used in selecting dose levels for chronic studies, if needed.

As a result of its broad spectrum of endpoints and observations, TG 407 is frequently part of regulatory data requirements. This pivotal study for the hazard assessment of chemicals is commonly performed for most high production volume chemicals, thus update of this Test Guideline suggested greater efficiency and lower animal use than constructing a battery of individual assays to address different mechanisms (VR, 2 to 5).

**PRP Comments**

The panel agreed that the updated test guideline (TG) 407 should not be considered to be a broad based screening assay for endocrine activity; the lack of confidence in negative results make this inappropriate. Only moderate or strong endocrine active substances (EAS) are identified (leading to 'false-negatives' for weak endocrine active substances (EAS)) and the validation exercise did not adequately

characterise the incidence of ‘false-positive’ responses. Regarding endocrine mediated effects, compound characterization should not therefore be based on the results of this TG alone but should use a weight-of-evidence approach using all existing data on a chemical to characterise potential endocrine activity. However, the TG was useful to identify strong or moderate EAS, and where other mechanisms such as metabolic modulation or toxicity or combinations of these with endocrine activity could cause endocrine disruption. Several members of the PRP characterised the TG as a broad, basic, repeat dose toxicity study.

There was considerable disagreement on whether the rationale was adequate. Whilst it was accepted that the frequent regulatory reference to TG 407 made an update important, including additional parameters, one member believed that the goals of the updated TG were now in conflict. This was because the update proposed differentiating between endocrinological effects which occur secondarily to toxic effects from specific disruptions to the endocrine system. One member believed that the rationale was outdated and failed to meet its rationale as it did not identify weak EAS; it could not be depended on to ‘red flag’ likely EAS.

While all the expectations for the updated TG 407 had not been met the panel agreed that the rationale would be acceptable if it clearly stated that it was not a broad-based screening assay that was likely to identify all EAS but represented a cost-effective means of effectively identifying moderate/strong EAS. This would be better than further refining the TG with the negative implications this would have for animal welfare.

**Criterion 2: The relationship between the test method's endpoint(s) and the (biological) phenomenon of interest should be described.**

This should include a reference to scientific relevance of the effect(s) measured by the test method in terms of their mechanistic (biological) or empirical (correlative) relationship to the specific type of effect/toxicity of interest. Although the relationship may be mechanistic or correlative, test methods with biological relevance to the effect/toxicity being evaluated are preferred.

**Current Status of the Validation (OECD Secretariat)**

In order to identify possible estrogens, antiestrogens, androgens, antiandrogens, and thyroid toxicants during the course of the 28-day repeat dose toxicity study, the principle for TG 407 updates is that the protocol incorporates the necessary array of endpoints sensitive to these mechanisms (VR, 6). Thus the updates of TG 407 rely on the measurement of the following parameters (see table below for more details):

Weight and histopathology of hormone-dependant tissues:

- male and female reproductive tracts
- thyroid and pituitary

Thyroid hormones (others not feasible)

Sperm and estrous parameters

**Comparison of the current TG407 endpoints with the proposed update endpoints investigated during the phase-2 studies (VR, Table 2)**

<b>Endpoint/ effects</b>	<b>Current TG 407 Endpoints</b>	<b>Proposed updates to TG 407</b>
Organ/tissue weights	liver, kidney, adrenals, testes, epididymides, thymus, spleen, brain, heart	<ol style="list-style-type: none"> <li>1. testes (each weighed separately)</li> <li>2. seminal vesicles + coagulating glands</li> <li>3. prostate (possible dissection and separate weights for ventral and dorsolateral prostate), ovaries</li> <li>4. thyroid</li> <li>5. uterus</li> </ol>
Histopathology	brain, spinal cord, stomach, small and large intestines, liver; kidneys, adrenals, spleen; heart, thymus, trachea, lungs, gonads, accessory sex organs (i.e., uterus, prostate), thyroid, urinary bladder, lymph nodes, peripheral nerve, bone marrow, all gross lesions	<ol style="list-style-type: none"> <li>1. pituitary</li> <li>2. vagina</li> <li>3. one epididymidis, seminal vesicles + coagulation glands</li> <li>4. mammary gland,</li> </ol>
Thyroid Hormones	none	<ol style="list-style-type: none"> <li>1. circulating levels of T<sub>3</sub> and T<sub>4</sub></li> <li>2. circulating levels of TSH</li> </ol>
Spermatology	none	<ol style="list-style-type: none"> <li>1. epididymal sperm number</li> <li>2. sperm morphology</li> </ol>
Estrous cycle	none	Daily vaginal smears to assess oestrous cycling via epithelial cytology for at least five days to ensure necropsy during diestrus

**PRP Comments**

The panel agreed that the selection of parameters during the validation exercise was transparent, related to biological phenomenon and scientifically sound. Several of the PRP members pointed out that the parameters used were all from existing TGs and that the TG builds on these parameters; this was considered to be an appropriate way forward as the parameters, their use(s), limitations, use in risk assessment etc were well understood. However some members of the PRP thought that whilst the end-points included were relevant they were not necessarily comprehensive. Other parameters would better take into account relevant sensitive life stages, species or exposure routes (such as developmental/prepubertal exposure, exposure of fish in the aquatic environment etc) but these are not compatible with an update of TG 407 which uses young adult rodents. The lack of additional controls, normally required for certain end-points, could lead to higher variability and therefore compromise the sensitivity of the end-points.

**Criterion 3: A detailed protocol for the test method should be available.**

The protocol should be sufficiently detailed and should include, *e.g.*, a description of the materials needed, such as specific cell types or construct or animal species that could be used for the test (if applicable), a description of what is measured and how it is measured, a description of how data will be analysed, decision criteria for evaluation of data and what are the criteria for acceptable test performance.

### **Current Status of the Validation (OECD Secretariat)**

A detailed protocol is provided, including guidance on the test materials needed: rat (age, weight, number of animals used), administration of doses, clinical observations, haematology and clinical biochemistry (emphasizing on conditions of sampling) and pathology.

General decision criteria for the evaluation of data are similar to existing OED Test guidelines, based on observation and statistical significance of measures. In addition, combination of TG 407 data with that from other studies (e.g. OECD reproduction toxicity screen) may be necessary in special cases (depending on exposure, use pattern etc.). A detailed guidance for histopathological evaluation of subtle effects and quality control procedures may also be needed to obviate increase in animal number (VR, 24, 276, 277, 338).

### **PRP Comments**

The panel agreed that the protocol was sufficiently detailed, for the most part. The level of detail was comparable to that in the current TG and was sufficiently detailed to perform the test (one member thought that additional clarifications were needed in the TG and these are recorded at Annex 1, table 1). One member believed that further details would be helpful for the in-life portion of the study to help understand the variables affecting hormone concentrations. Two members of the group believed that the group size is too small to detect changes with weak acting compounds because of the physiological variability in hormone concentrations.

The panel agreed that greater guidance and further clarification was needed on data interpretation. For example, how are non-statistically significant (but numerical changes) alterations in the end-points interpreted, how to distinguish between effects that are consistent with endocrine disruption from non-specific effects that are not related to potential endocrine disruption, what changes constituted 'positive' versus 'negative' effects (e.g. weight changes for body-weight independent organs), additivity of effects in a number of organs?

### **Criterion 4: The intra- and inter-laboratory reproducibility of the test method should be demonstrated.**

Data should be available revealing the level of reproducibility and variability within and among laboratories over time. The degree to which biological variability affects the test method reproducibility should be addressed.

### **Current Status of the Validation (OECD Secretariat)**

In phase-2, for each chemical, duplicate independent studies have been performed in 2 laboratories, for a total of 20 studies (VR, 317). Overall, the TG 407 data and findings generally were in good agreement between the two laboratories conducting studies on the same substance. The findings in these studies support the reproducibility and reliability of the traditional use of the TG 407 in flagging possible systemic and target organ toxicity. For each of the ten test substances, changes in body weights, haematology and clinical chemistry parameters, and current absolute and relative tissue weight endpoints, and the histopathology endpoints have been compiled into comparative tables in each of the test substance chapters of the validation report. In addition, where other toxicological data were available on the test substance or a close mechanistic relative, these same endpoints have been compared with TG 407 data at the end of each test substance chapter.

These comparisons show that, within the TG 407 studies and with other toxicological data, these endpoints are fundamentally reproducible and that the TG 407 is basically a reliable predictor for other studies. The changes in body weight were a reproducible effect between the TG 407 studies, noting that, based on the power calculations, the percent change in body weight often had to be > 10% to achieve significance even with the low CV for this measurement. Similar body weight changes were observed in longer and more detailed toxicological assays. For haematological and clinical chemistry parameters, four basic classifications were entertained: 1) the parameters was statistically significant in both studies, 2) the parameter was statistically significant in one study and the absolute trend in the other study was in agreement, 3) the parameters was statistically significant in one study and the absolute trend in the other study was unchanged or moved in the opposite direction, and 4) the direction of statistical significance was in the opposite direction between the two studies. Within the TG 407 studies, the bulk of the changes fell into the first two categories. These data were often not published for other toxicological assays, so no comparison here was consistently made and no conclusion is offered. For tissue weights, such as liver, kidneys, and adrenals, the TG 407 studies were fundamentally reproducible and matched findings in longer and more detailed toxicological assays. Histopathological findings were also similar both among the TG 407 studies for a test substance and with longer and more detailed histopathological studies. Due to the longer time of administration, LOEL doses in these non-TG 407 studies were typically lower, as would be expected (VR, 336, 337).

The CV values of the proposed update tissue weights were slightly higher than a number of current tissues. For example, current tissues, that are larger and easier to dissect, such as liver and testes, had lower mean CVs ranging from about 8 to 11. As tissues became smaller and more difficult to dissect, CVs increased. Mean CVs were 12-14 for paired adrenals, 18-19 for pituitary, and 20-21 for the thyroid. Fluid-filled male accessory tissues that are difficult to dissect, such as the prostate lobes, had higher mean CVs approaching 24. The CVs for the uterus and for the male accessory tissues were consistent with those observed in the uterotrophic and Hershberger validation programs. In addition, as also with the uterotrophic and Hershberger studies, CV values varied and appeared to be related with the individual performing laboratories. This suggests that laboratory technique is a possible variable and could be connected to the ability to detect weakly acting substances (VR 338).

### **PRP Comments**

The panel agreed that the criterion was sufficiently met for an animal test. Several members pointed out that the variability from using animals in 'small' subgroups contributed to the inability of the TG to detect weak acting EAS; the more potent substances were more readily detected by statistically-significant changes in the end-points. One member thought that the lack of blind coding of tests could potentially have an influence.

**Criterion 5: Demonstration of the test method's performance should be based on the testing of reference chemicals representative of the types of substances for which the test method will be used.**

A sufficient number of the reference chemicals should have been tested under code to exclude bias.

**Current Status of the Validation (OECD Secretariat)**

**Representativity**

For phase-2 of the validation, consultation by the Lead Laboratory with the participating laboratories and other experts led to the proposal for a battery of ten test substances, ranging from pharmaceuticals to substances with broad environmental release and human exposure. These substances, covering a large range of modes of action (see table below) include 6 strongly acting substances and 4 substances that are considered to be weakly acting. The six strongly acting substances were ethinyl estradiol, tamoxifen, methyl testosterone, flutamide, l-Thyroxine, and propylthiouracil, and the four additional weak substances were CGS 18320B, *p,p'*-DDE, genistein, and nonylphenol.

<b>Chemical</b>	<b>Mode of action</b>	<b>Type of chemical</b>	<b>Testing phase of the updated TG 407 validation study</b>
Ethinyl estradiol	Potent oestrogen	Pharmaceutical	Tested in phase-1 and 2
Genistein	Weak oestrogen	Natural isoflavone	Tested in phase-2
Nonylphenol	Weak oestrogen	Industrial chemical	Tested in phase-2
Tamoxifen	Potent antioestrogen	Pharmaceutical	Tested in phase-1 and 2
CGS 18320B	Antioestrogen - Aromatase inhibitor	Metabolic inhibitor	Tested in phase-2
Methyltestosterone (MT)	Potent androgen (aromatisable)	Pharmaceutical	Tested in phase-1 and 2
Flutamide (FLU)	Potent anti-androgen	Pharmaceutical	Tested in phase-1 and 2 (reference chemical in phase-1)
<i>p,p'</i> -DDE (DDE)	Weak anti-androgen	Pesticide	Tested in phase-2
Methoxychlor	Weak estrogen - antiandrogen	Pesticide	Tested in phase-2
Propylthiouracil	Thyroid toxicant	Pharmaceutical	Tested in phase-1 and 2
l-Thyroxine	Thyroid agonist	Natural hormone, Pharmaceutical	Tested in phase-2

**Coding**

In Phase-1 and Phase-2, none of the substances tested has been coded. The purpose of coded substance is to avoid bias in the results due to prior knowledge of the expected responses. Guidance Document 34 says that it is “preferable” but it is not a requirement.

### **PRP Comments**

The PRP agreed that the number of reference chemicals was small but that this was acceptable for reasons of animal welfare and also because the TG was being updated rather than developed from scratch so the various parameters could be regarded as having been validated. Several of the PRP thought that whilst non-blind reading was a theoretical problem, in practice it was not because of the level of complexity involved and whilst no negative reference chemical was used, with certain limitations, compounds representing one mode of action can be considered as a negative reference for other modes of action. Several of the PRP would have liked weak EAS to have been used as reference chemicals in addition to those used. One member pointed out that no weak-acting thyroid agents were evaluated (DDE which affects the thyroid via liver enzyme induction as Phenobarbital could be considered to be a surrogate).

### **Criterion 6: The performance of the test method should have been evaluated in relation to relevant information from the species of concern, and existing relevant toxicity testing data.**

In the case of a substitute test method adequate data should be available to permit a reliable analysis of the performance and comparability of the proposed substitute test method with that of the test it is designed to replace.

### **Current Status of the Validation (OECD Secretariat)**

In the case of the updated TG 407, the new parameters incorporated to the TG are intended to enable an initial screening of effects (endocrine disruption potential) that are not investigated in the current TG 407. Thus a comparison between current TG 407 and updated TG 407 would not be relevant.

However, in phase-2, the laboratories were requested to conduct the full updated TG 407 so as to assess whether any interference or compromise would be encountered with the functional observation battery or any other current protocol requirements (VR, v). The validation study has proved that the ability of laboratories to perform the entire TG 407 protocol was not negatively impacted by the updates. Laboratories were able to conduct the functional observation and motor activity batteries without interference (VR, xii).

A comparison has been made in most cases between the results of the updated TG 407 and other toxicological assays with longer and or *in utero* exposures, with the same or similar endocrine active test substances. At least some data were found for all substances except methyl testosterone and l-thyroxine. These comparisons support the ability of the TG 407 to detect systemic, target organ and endocrine-related toxicities for potent chemicals. Where chronic studies or reproductive and development studies easily detected effects, concordant results were typically found in the current TG 407 studies for systemic, target organ, and endocrine toxicities, but where only marginal effects were observed in longer term studies, the updated TG 407 may not be able to detect clear endocrine-related effects.

As expected, the chronic circumstances in the reproductive and developmental studies usually resulted in the findings at lower doses than in the TG 407 studies, but not in all cases such as with PTU. These comparisons are summarised at the end of each test substance chapter in the validation report (VR, 343).



**PRP Comments**

The PRP agreed that the criterion was met. One member believed that the analysis was inadequate as it did not consider the results from ongoing validation work on OECD projects and draft test guidelines and assays in peer review. One member thought that the assessment of the oestrus cycle and killing of females in diestrus could be further considered N.B. assessment of the cycle for at least 5 consecutive days and killing in diestrus is contained in draft updated TG 407.

**Criterion 7: Ideally, all data supporting the validity of a test method should have been obtained in accordance with the principles of GLP.**

Aspects of data collection not performed according to GLP should be clearly identified and their potential impact on the validation status of the test method should be indicated.

**Current Status of the Validation (OECD Secretariat)**

The laboratories were encouraged to perform the updated TG 407 protocol in compliance with Good Laboratory Practices (GLP). From the reports received from the various laboratories, it appeared that 13 of the 20 studies were carried out in full compliance with GLP. Five of the studies were carried out under procedures that could be considered as generally complying with GLP, but that would require a quality assurance audit for one or more aspects in order to bring the studies into full, confirmed compliance. Two studies were apparently not carried out in accordance with GLP.

The GLP status of the studies is summarised at Annex 1, and additional comments have been provided as to whether the test substance dosing solutions were analyzed to confirm that the nominal doses were indeed administered. This important study element was confirmed to have been done for 18 of the 20 updated TG 407 studies (VR, 24, 25, table 8).

**PRP Comments**

The PRP agreed that this criterion was met. Where data was not collected in accordance with GLP there appeared to be no impact on the validity of the results.

**Criterion 8: All data supporting the assessment of the validity of the test method should be available for expert review.**

The detailed test method protocol should be readily available and in the public domain. The data supporting the validity of the test method should be organised and easily accessible to allow for independent review(s), as appropriate. The test method description should be sufficiently detailed to permit an independent laboratory to follow the procedures and generate equivalent data. Benchmarks should be available by which an independent laboratory can itself assess its proper adherence to the protocol.

**Current Status of the Validation (OECD Secretariat)**

It is intended that the materials documenting the protocol development, validation and associated supporting documents will be made freely available by promulgation through the OECD or by publication in the peer reviewed literature. The validation report has been declassified by the Joint Meeting and is now available on the OECD public website. Additionally, a publication to compare the results obtained with the enhanced 407 with data from standard tests of longer or *in utero* exposure has been written on behalf of CEFIC Endocrine Modulators Steering Group (CEFIC-EMSG). It has now been accepted for publication

in a scientific journal. A draft of the Test guideline is also available to the Peer reviewers on the OECD public website.

### **PRP Comments**

The PRP agreed that this criterion was met. The panel agreed that further guidance and training would be needed (e.g. trimming of tissues, histopathology).

### **Conclusion**

Has the updated TG 407 been sufficiently evaluated and has its performance been satisfactorily characterized by the OECD validation program to support its proposed extended use for also screening the potential of substances to act as (anti)estrogens, (anti)androgens or thyroid toxicants *in vivo*?

### **PRP Comments:**

The PRP agreed that the TG was a useful update of the existing TG 407 and addition to the bank of TGs. However, its lack of sensitivity ('false-negatives') means that it is not appropriate to use as a 'classical' screening assay. The problems with identification (or lack) of weak EAS and the unknown rate of 'false-positives' (N.B. difficult to identify and assess) mean that the results must be used with care. The panel stressed that regulatory decision making on endocrine activity (compound characterisation) should not be solely based on results from application of this TG. Several members pointed out that basing a screen on adult animals is unlikely to result in a sensitive screening assay.

The panel however agreed that the addition of end-points to the current TG 407 would enhance the ability of the updated TG 407 to detect strong or moderate EAS. As part of a weight of evidence approach the updated TG would also in some cases provide additional information on possible health hazards from repeated exposure over a limited period of time, flag possible systemic and target organ toxicity, and provide first evidence of dose response relationship.

The panel believed that only moderate or potent EAS would be detected and therefore the updated TG 407 cannot be considered to be a 'stand-alone' test for detecting EAS. A negative result cannot be accepted as meaning that a substance is not an EAS nor can a positive result be taken as categorical proof that a substance is an EAS. Additional assays are needed to detect weak EAS; some assays already exist to identify some weak acting EAS and it is expected that new approaches will be developed and validated in the near future. One member pointed out that weak-acting thyroid active agents were not evaluated and may not be detected (as mentioned above, DDE could be considered to be a surrogate, in which case a weak activating compound is also included).

In conclusion, the panel agreed that whilst there were shortcomings in the updated TG 407 it was a useful update, at least in the short to medium term, to the battery of test guidelines. These shortcomings need to be clearly stated as well as greater guidance provided to those operating the TG.

**ANNEX 1 - DETAILED COMMENTS FROM PRP MEMBERS ON TECHNICAL ISSUES**

Two members recommended performing counts of homogenization-resistant spermatids (testis) rather than sperm from the epididymis as the former is associated with a lower variability than sperm counts.

One member thought it was inappropriate to require “at least 2 doses below the overt toxic dose” (para 14.a) as even with range-finding studies the dose selection cannot guarantee that major toxicity is not seen in a lower dose group. This could mean that a request to have 2 doses below major toxicity would require a further study (even if the overall evaluation of the toxicity profile is possible). A change of wording to make clear that overt toxicity should be limited to the highest dose tested, as far as is reasonably possible, should be considered.

One member believed that the updated TG should require *in vitro* testing for endocrine activity before looking at endocrine effect associated parameters; this would require a definitive scheme of testing and assessment to be developed and stipulated in advance. Another member pointed out that not all substances having endocrine activity can be detected by *in vitro* systems due to the metabolism of parent compounds and possible effects on the metabolism and synthesis of endogenous hormones.

One member proposed the following detailed changes to updated TG 407:

<b>Table 1</b>		
<b>Issue</b>	<b>Position</b>	<b>Justification</b>
The timing of female necropsy needs to be adjusted by oestrous cycle.	Females in the same group should be necropsied on the same day relative to their oestrous cycles.	It enables the same level of observation for histopathological examinations (uterus, vagina, mammary gland).
Measurement of thyroid hormone (T4) (draft TG: 29a, 29b)	Not necessary for T4 measurement	T4 data showed wide variations in the validation studies.
Measurement of thyroid hormone (plasma) (draft TG: 29b)	Change "plasma samples" to "plasma or serum samples".	1. In examination of clinical biochemistry, they usually use serum samples. 2. Thyroid hormones are able to be measurable with serum samples as well as plasma ones. 3. If samples are limited to plasma only, it takes more time and work because a separate blood sample will have to be collected for the plasma sample.
Measurement of thyroid hormone	Is measurement of thyroid hormones necessary?	In the validation studies, histopathological examination was more sensitive than thyroid hormone measurement. Effects on thyroids could be one of the histopathological findings.
Observation of oestrous cycle by vaginal smear (draft TG: 23a)	Synchronization of the oestrous cycle by vaginal smear is not necessary for blinded examination.	Technically, it is not difficult to collect and evaluate vaginal smears, regardless of the cycle phase.
Fixation of tissues such as testis, epididymis	Appropriate fixation (ex. Bouin's solution etc.) needs to be selected.	1. Good specimens are not always obtained from formaline fixation. 2. Testis is an important target organ of toxic substances. 3. As there are no other endpoints related to testicular toxicity, histopathological examination of testis is very important.
Diet sample retention and analysis of dietary contaminants (draft TG: 8a)	Is this necessary? If needed, which contaminants should be tested for, and how does one determine their standard levels? A detailed protocol needs to be provided.	1. This updated TG407 can not detect weak hormone active substances, making analysis of trace amounts of dietary hormone like substances questionable. 2. Since there is no protocol for testing for dietary contaminants in the current TG, some confusion will occur unless a detailed protocol is available.
Additional parameters of blood clinical biochemistry (draft TG: 29)	A specific description for additional parameters needs to be provided.	This TG is an assay for evaluating endocrine affecting substances.

## ANNEX 2 - GOOD LABORATORY PRACTICE (GLP) COMPLIANCE OF THE 407 STUDIES.

Chemical	Lab	GLP Compliance		Comments on GLP and substance and/or dosing sample analyses
		Yes	No	
Ethinyl Oestradiol	2	✓		EE stock and dosing solution analyses were conducted and reported. For technical reasons, the dosing solutions were analysed at an outside, non-GLP compliant laboratory.
	5	✓		EE stability and stock and dosing sample analyses were stated to have been done, but specific results were not reported.
Genistein	4	✓		Genistein stock and dosing sample analyses were conducted to confirm levels, and the results were reported.
	12	✓		Genistein stock and dosing sample analyses were conducted to confirm levels, and the results were reported.
Nonylphenol	1	✓		Nonylphenol stock and dose sample analyses were conducted during the study, and results were reported.
	6	✓		Nonylphenol stock and dosing samples analyses were conducted during the study, and the analytical results were reported.
Tamoxifen	3		✓	According to the final report: "This study was not performed in compliance with Good Laboratory Practice in that it was not subjected to specific Quality Assurance inspections. It was performed according to standard operating procedures which were previously accepted and periodically inspected by Quality Assurance Unit."
	10		✓	The report did not state whether tamoxifen dosing sample analyses were done, and no analytical results were reported.
CGS 18320B	8	✓		CGS 18320B analyses for the test substance stability and dosing samples were conducted to confirm dosage levels, and the analytical results were reported.
	13	✓		CGS 18320B stock and dosing samples analyses were conducted to confirm dosage levels, and the analytical results were reported.
Methyl Testosterone	3		✓	According to the final report: "This study was not performed in compliance with Good Laboratory Practice in that it was not subjected to specific Quality Assurance inspections. It was performed according to standard operating procedures which were previously accepted and periodically inspected by Quality Assurance Unit."
	12	✓		Methyl testosterone stock and dosing sample analyses were conducted to confirm dosage levels, and the analytical results were reported.
Flutamide	2	✓		Flutamide stock and dosing solution analyses were conducted to confirm dosage levels and the analytical results were reported.
	11	✓		The report did not state whether Flutamide dosing sample analyses were done, and no analytical results were reported.
<i>p,p'</i> -DDE	6	✓		DDE test substance dosing samples were analysed for homogeneity and stability during the study, and the analytical results were reported.
	7		✓	DDE stability and test substance dosing samples were analysed, and the results were reported.
Propyl-thiouracil	1	✓		PTU test substance dosing samples were analysed during the study, and the results were reported.
	10		✓	The report did not state whether PTU dosing sample analyses were done, and no analytical results were reported.
l-Thyroxine	9		✓	Thyroxine test substance dosing samples were analysed, and the analytical results were reported.
	13	✓		Thyroxine analyses for the test substance dosing samples were conducted to confirm dosage levels, and the analytical results were reported.