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

Draft Summary Record of the Meeting of the Fish Drafting Group

21-22 October 2008, OECD Headquarters, Paris, France

Anne GOURMELON
Tel: +33 (0)1 45 24 98 49; Fax: +33 (0)1 44 30 61 80; Email: anne.gourmelon@oecd.org
Opening of the meeting and objectives

1. The meeting of the Fish Drafting Group was held at the OECD Headquarters, 2 rue André-Pascal, Paris on 21 and 22 October 2008. The meeting was attended by 15 participants from Denmark, Germany, Japan, Switzerland, the United Kingdom, the United States, and the European Commission and BIAC. The Secretariat, Anne Gourmelon, chaired the meeting.

2. The Secretariat reminded participants of the main objective of the meeting which were 1) to review comments and issues raised in relation to the draft Test Guideline on the Fish Screening Assay and propose changes and solutions as appropriate, and 2) to review and comment on the proposal for Phase 2 of the validation of the Fish Sexual Development Test, led by Denmark.

Adoption of the draft agenda

3. Before the meeting, John Green, BIAC, had provided a paper on the statistical power of endpoints in relation to the draft Test Guideline on the Fish Screening Assay. Ioanna Katsiadaki, United Kingdom, had requested some time during the meeting to present progress with the validation of the stickleback assay for the screening of anti-androgenic chemicals. Les Touart (United States) made a presentation of the Fish Short-Term Reproduction Assay peer review via-à-vis the 8 validation criteria of the OECD Guidance Document 34. All slides presented at the meeting were made available on the meeting site.

4. The draft agenda was approved without further changes.

Issues associated with the draft TG on the Fish screening assay, identified from the comments received

5. The meeting discussed the value of having a list of chemicals, endpoints and effect levels as an Annex to the Test Guideline. Although initially some participants thought it was a good idea to have such a list for information, others thought it was cumbersome and should rather be addressed by a list of chemicals for the technical proficiency of the laboratory. It was agreed that a paragraph will be developed in the Test Guideline making reference to a list of chemicals covering a range of modes of action for laboratories performing the test for the first time.

Validity criterion on mortality

6. The meeting agreed to revise the text to: “the mortality in the water (or solvent) controls should not exceed 10 per cent at the end of the exposure period”, and to remove reference to the treatment levels.

Guidance on quantitative criterion for reproductive activity
7. In the draft Test Guideline, revised just before the meeting following comments received in September 2008, a number of about 15 eggs/female/day over a 4-day during the pre-exposure period was proposed for fathead minnow, as a guide (paragraph 19 of the draft TG), derived from experience with fathead minnow studies from the United States. The meeting agreed that there was a need to consider existing data before providing a quantitative criterion, possibly leading to study rejection, or even simple guidance. However, there was concern that not all laboratories could achieve this number. For other species than fathead minnow, this approximate number could also serve as a guide, however not as a hard criterion. The meeting recommended that Les Touart (United States) should investigate how this number was derived as a criterion for the US assays. Mark Jaber (United States) agreed to investigate fathead minnow studies conducted at Wildlife International [following the meeting, Mark Jaber informed the Secretariat via email that the studies conducted at Wildlife International yielded more than 15 eggs/female/day, thus not an issue anymore]. Concerning the issue of robustness of the fecundity endpoint, John Green, BIAC, agreed to review the statistical power of the fecundity data, based on data collected in Phase 1B of the validation, and also based on existing data collected from fathead minnow studies using the same test design as in Phase 1B. The group will be advised of the outcome of this investigation in a teleconference in November.

**Inclusion or not of zebrafish as a test species**

8. The experts in zebrafish were of the opinion that the differentiation between males and females is not a problem in adult fish, and they recommended that zebrafish should be kept as a test species especially if fecundity and gonad histopathology are additional endpoints in the assay.

**Inclusion of positive controls other than estrogen**

9. After discussing the various combinations for running positive control substances, either one each time the assay is performed, or one every now and then, and other possibilities, participants agreed that there was no need for a positive control. However, the meeting recommended that proficiency chemicals (one for each important mode of action known) be demonstrated by laboratories performing the assay for the first time or performing the assay after a substantial change in the laboratory (e.g. change of fish strain or supplier). This concept was developed in the section on Guidance for the interpretation and acceptance of the test results.

**Dose setting: should the highest dose be 1/3 or 1/10 of the LC50? Or recommend range-finding study?**

10. Participants were of the opinion that while it is difficult to set the highest dose, 1/10th of the LC50 seemed more appropriate from experience. However, because LC50 data may not be easily available for all chemicals, the meeting agreed to revise the text to recommend a range-finding study or the use of other ecotoxicity data to set the maximum tolerated concentration.

**Guidance on when to perform gonad histology**

11. The meeting agreed that the current guidance provided in the last two paragraphs of the draft TG were appropriate and did not recommend making changes.

**Other issues identified**

12. John Green, BIAC, presented the outcome of the power analyses on the vitellogenin and sex characteristics endpoints. For vitellogenin, the conclusion was confirmed that the endpoint yields sufficient statistical power under the proposed test design for both males and females measurements. For the sex characteristics, he indicated that male measurements yields low power, due to variability observed in the data sets. For female, no calculation of statistical power was undertaken. Presumably unexposed female
having no such sexual characteristics and power calculation being based on analysis of variability, no such calculations can be performed. The background document on the statistical power is available on the meeting website.

13. In relation to the use of solvents, a text proposal from James Wheeler, BIAC, for former paragraphs 17 and 53 was reviewed by the meeting and agreed with some changes.

**Revision of the draft Test Guideline**

14. The meeting went through the revised draft Test Guideline, paragraph by paragraph and agreed on a number of changes and annotations. It was agreed in particular that for the fecundity endpoint, annotations should make reference to the fact that power analyses are in progress to investigate the power of this particular endpoint.

15. In relation to the annexes of the Test Guideline, participants agreed that there were several instances where too much detail was provided and should be removed. The Secretariat will review the annexes and remove excess details.

**Status of the Guidance Document on the Fish Gonad Histopathology**

16. After reading the draft guidance document on gonadal histopathology, pathologists participating to the meeting realised that the version available was not reflecting the entirety of the work that had gone into this document, in particular, the guidance on histology preparation was not there, and the specific recommendations from the Heidelberg workshop (November 2004) were lost in the document and needed to be set out more clearly up front. Les Touart, United States, agreed to have a closer look at the document. Thomas Braunbeck, Germany, indicated that he would also have a look at the document once revised. The guidance document will then be circulated to the pathologists involved in the drafting of the document and in the histopathology work in Phase 1A and Phase 1B for a quick review before sending to the National Coordinators.

**Next steps and timelines**

17. The Secretariat confirmed the next steps:

   - Investigation of the statistical power of the fecundity endpoint (John Green), through analysis of data sets from the three fish species collected during Phase 1B of the validation study and similar studies on the fathead minnow short term reproduction assay performed in the United States (Secretariat and Les Touart);
   - Revision of the draft guidance document on the fish gonadal histopathology (Les Touart/Thomas Braunbeck);
   - Revision of the draft Test Guideline based on changes agreed at the meeting (Secretariat);
   - Teleconference of the Fish Drafting Group the fourth week of November to review robustness of the fecundity endpoint and progress with the revised draft TG and the draft guidance document on gonadal histopathology.

**Work on the validation of the Fish Sexual Development Test**

18. Henrik Holbech and Gitte Petersen (Denmark) presented the proposal for Phase 2 of the validation of the Fish Sexual Development Test. Participants generally agreed with the details of the
proposal. There was discussion on the choice of the androgenic chemical proposed, i.e. methyl dihydrotestosterone (MDHT). Henrik Holbech indicated that there was no analytical method yet available for the determination of test concentration, and that was a task he was going to work on. John Green, BIAC, will advise on the number of fish to be used for the medaka and the stickleback where the genetic sex is determined as well as the phenotypic sex.

19. A teleconference will be organised at the end of November to address the protocol details. All participating laboratories will be requested to attend the teleconference. The time schedule for Phase 2 will be defined after the teleconference; each laboratory should indicate their time plans.

Any other business

20. Ioanna Katsiadaki (United Kingdom) presented progress with the stickleback assay for anti-androgen detection. The work was well received by participants who really saw the value of the assay for detecting/confirming the anti-androgen mode of action. She indicated that manuscripts had already been published and other(s) were in preparation.

21. The meeting was closed at 16h45 on Wednesday 22 October.
ANNEX 1

List of participants

Denmark

Dr. Henrik HOLBECH
University of Southern Denmark, Odense
Institute of Biology
Campusvej 55
5230 Odense M
Denmark

Tel: +45 6550 2770
Fax: +45 6593 0457
Email: hol@biology.sdu.dk

Ms. Gitte PETERSEN
Senior Biologist, Ph.D.
Department of Environmental Risk Assessment
DHI - Water & Environment
Agern Allé 11
DK-2970 Hørsholm
Denmark

Tel: +45 45 16 93 12
Fax: +45 45 16 92 92
Email: gip@dhi.dk

Germany

Professor Thomas BRAUNBECK
Department of Zoology
University of Heidelberg (UNI)
Neuenheimer Feld (INF) 230
69120 Heidelberg
Germany

Tel: +49-6221-54-5668
Fax: +49-6221-54-6162
Email: braunbeck@zoo.uni-heidelberg.de

Dr. Reinhard LAENGE
Ecotoxicologist
LGE, Ecotoxicology
Bayer Schering Pharma AG
13342 Berlin
Germany

Tel: +49 30 468-15328
Email: reinhard.laenge@bayerhealthcare.com
Dr. Christoph SCHAEFERS  
Ecotoxicology  
Fraunhofer Institute for Molecular Biology and Applied Ecology  
Auf dem Aberg 1  
57392 Schmallenberg  
Germany  
Tel: +49 2972 302 270  
Fax: +49 2972 302 319  
Email: christoph.schaefers@ime.fraunhofer.de

Dr. Taisen IGUCHI  
Professor  
National Institute for Basic Biology, National Institutes of Natural Sciences  
Okazaki Institute for Integrative Bioscience  
5-1 Higashiyama  
Myodaiji  
444-8787 Okazaki  
Japan  
Tel: +81(564) 59-5235  
Fax: +81(564) 59-5236  
Email: taisen@nibb.ac.jp

Mr. Chisumi ETO  
Manager  
Chemical Assessment Center  
Chemicals Evaluation and Research Institute  
1-4-25 Kouraku, Bunkyo-ku  
112-0004 Tokyo  
Japan  
Tel: +81(3) 5804-6136  
Fax: +81(3) 5804-6139  
Email: eto-chisumi@ceri.jp

Dr. Norihisa TATARAZAKO  
Senior Researcher  
Research Center for Environmental Risk  
National Institute for Environmental Studies  
16-2 Onogawa, Tsukuba  
305-8506 Ibaraki  
Japan  
Tel: +81 (29) 850-2887  
Fax: +81 (29) 850-2851  
Email: tatarazako.norihisa@nies.go.jp
Switzerland

Dr. Hans RUFLI
ecotoxsolutions
Schwarzwaldaallee 215
CH-4058 Basel
Switzerland

Tel: +41 61 272 33 20
Email: rufli@ecotoxsolutions.com

United Kingdom

Dr. Ioanna KATSIADAKI
Weymouth Laboratory
CEFAS
Barrack Road The Nothe
Weymouth
United Kingdom

Tel: +44 1305 206 600
Fax: +44 1305 206 601
Email: ionna.katsiadaki@cefas.co.uk

United States

Mr. Mark JABER
Wildlife International Ltd
8598 Commerce Drive
Easton
United States

Tel: +1 410 822 8600
Fax: +1 410 822 0632
Email: MJABER@WILDLIFEINTERNATIONAL.COM

Dr. Leslie TOUART
OPPTS/Office of Science Coordination and Policy
US Environmental Protection Agency
1200 Pennsylvania Ave NW (7304M)
20460 Washington
United States

Tel: +1 202 564 8468
Fax: +1 202 564 8483
Email: touart.les@epa.gov

EC

Ms. Miriam JACOBS
Institute for Health and Consumer Protection (IHCP)
ECVAM
European Commission - Joint Research Centre
Via E. Fermi 279
210217 Ispra
Italy

Tel: +39 0332 78 6512
Fax: +39 0332 78 5845
Email: miriam.jacobs@jrc.it
Business and Industry Advisory Committee (BIAC)

John W GREEN
Senior Consultant: Biostatistics
DuPont Applied Statistics Group
Stine-Haskell Research Center
Bldg H-1 Rm 423B
1090 Elkton Road
P.O. Box 50
19714-0050 Newark
United States

Tel: +1 302 366 5310
Fax: +1 302 366 5207
Email: John.W.Green@usa.dupont.com

Dr. James WHEELER
Environmental Safety
Syngenta
Syngenta Ltd
Jealott's Hill International Research Centre
RG42 6EY Bracknell
United Kingdom

Tel: +44 (0) 1344 41 43 84
Email: james.wheeler@syngenta.com

OECD

Mme Anne GOURMELON
Administrator, Test Guidelines
ENV/EHS
OECD
Annexe Maillot 5029
2 rue André-Pascal
75016 Paris
France

Tel: +(33-1) 45 24 98 49
Fax: +33 1 44 30 61 80
Email: Anne.GOURMELON@oecd.org

Ms. Ciara MULLER
Assistant
ENV/EHS
OECD
Annexe Maillot 5055
2 rue André-Pascal
75016 Paris
France

Tel: +(33-1) 45 24 16 74
Email: Ciara.MULLER@oecd.org
Mme Laurence MUSSET
Principal Administrator
ENV/EHS
OECD
Annexe Maillot 5047
2 rue André-Pascal
75016 Paris
France
Tel: +(33-1) 45 24 16 76
Fax: +33 1 44 30 61 80
Email: Laurence.MUSSET@oecd.org