Initiatives Abroad and in Japan

Current OECD Initiatives and Progress in Endocrine Disrupters Testing and Assesement

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In 1998, the OECD launched a Special Activity on Endocrine Disrupter Testing and Assessment. This activity was initiated at the request of the Member countries and the Business and Industry Advisory Committee to the OECD (BIAC) to ensure that testing and assessment approaches for endocrine disrupters would not substantially differ among countries. In order to manage the work of the Special Activity, the Task Force on Endocrine Disrupters Testing and Assessment (EDTA) was established in the same year.

Initially the focus was on the development of new and the updating of existing test methods. The development of selected tests as OECD Test Guidelines involved formal validation of these tests. To that end Validation Management Groups were established for the validation of mammalian tests (VMG-Mammalian) and for ecotoxicity tests (VMG-eco), respectively. In June 2002, the EDTA agreed that a third VMG was needed for the validation of *in vitro* and other non-animal tests (VMG-*in vitro*). Current test method development and validation activities include: the uterotrophic assay, the Hershberger assay, the enhancement of Test Guideline 407, fish screening and full life cycle tests and reproduction test(s) in birds (for the screening and testing of (anti)estrogens and (anti)androgen effects, amphibian metamorphosis test (for thyroid hormone function disruption), and invertebrate tests.

In order to provide a framework for the testing and assessment of potential endocrine disrupters in which the various newly developed tests would fit, a conceptual framework was developed in 1998. It was intended to apply to both new and existing substances and for different chemical sectors including pharmaceuticals, industrial chemicals and pesticides. The conceptual framework has been substantially revised by the EDTA in its June 2002 Meeting in Tokyo. It was confirmed that it is not a testing scheme but rather a toolbox in which the various tests that can contribute information for the detection of the hazards of endocrine disruption are placed.

Considering the vast amount of work on endocrine disrupters, it was considered appropriate to find ways for sharing (at least some of) the work internationally. In June 2001, Member countries agreed that work should start to co-ordinate sharing the actual testing of existing substances for possible endocrine disrupting activities and to find ways to share assessments of tested chemicals. Member countries further agreed that the work-sharing activity should start as a small activity that could gradually increase if proven successful. First priority would include the grouping of chemicals of interest, sharing information and test results of High Throughput Screens and exchanging information on ongoing animal tests. Sharing of assessment reports of specific substances was also considered as extremely useful. This work has now begun and will be expanded in coming years.

Global Assessment of the State-of-the-science of Endocrine Disruptors

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In 1997, the International Programme on Chemical Safety (IPCS) was requested by the Intergovernmental Forum on Chemical Safety and the 1997 Declaration of the Environment Leaders of the Eight (G8) on Children's Environmental Health to undertake a global assessment of the current state of scientific knowledge relative to environmental endocrine disruption. This request was endorsed by the 50th World Health Assembly in 1997. The report, which was published in August 2002 and entitled "Global Assessment of the State-of-the-Science of Endocrine Disruptors", is the result of a global comprehensive review of the publicly available scientific literature on EDCs organized by IPCS, a collaborative programme of the World Health Organization (WHO), the United Nations Environment Programme (UNEP) and the International Labour Organization. Over 60 international scientific experts provided input into this document either as IPCS Steering Group Members, chapter leaders, authors, or reviewers. The assessment is unique in providing a global perspective on the endocrine disruptor issue, and in providing a framework by which strength-of-the-evidence analysis can be performed to determine whether there is a causal association between an adverse biological effect and exposure to an endocrine disrupting chemical (EDC). The report concludes that there is sufficient evidence that adverse effects have occurred as a result of exposure to EDCs in some wildlife species, though the evidence that human health has been adversely affected is generally weak. Therefore, because of continuing concerns and scientific uncertainties, studies on the potential effects posed by these chemicals should remain a high global priority requiring coordinated and strengthened international research strategies. There is, in particular, an urgent need for studies in vulnerable populations, and especially in infants and children, since exposure during critical developmental periods may have irreversible effects. Financial support for this project was provided by the Government of Australia, Health Canada, the European Commission, the German Ministry of Environment, the Japanese Ministry of Health, Labour and Welfare, the Institute of Public Health, Norway, the Swedish Chemicals Inspectorate, the Swedish Environmental Protection Agency, the Swedish Foundation for Strategic Environmental Research, the UK Department for Environment, Food and Rural Affairs, the UK Department of Health, the US Environmental Protection Agency, and the US National Institute of Environmental Health Sciences.

Testing for Endocrine Effects in Waste Water

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Domestic and industrial waste waters may have adverse effects on reproduction in fish and invertebrates. Effluent water from a variety of sources may contain substances of natural as well as anthropogenic origin that interfere with the endocrine metabolism of aquatic animals. Many of these endocrine disrupting chemicals resemble steroid hormones, and have therefore the potential to adversely disturb sexual development in exposed fish. Skewed sex ratios or inter-sex characteristics, such as the occurrence of both testicular and ovarian tissue in the same gonad, have been found in several fish species. In the development of screening tests for endocrine disruption in fish, so far, tropical fish have been given priority. In a Swedish project we use the three-spined stickleback, *Gasterosteus aculeatus*, as a model organism. This small-sized teleost fish can be considered as a representative for waters in temperate climate zones in the northern hemisphere. It reproduces in the whole range from sea to freshwater. Since it spawns in laboratory, the stickleback can be studied at each stage of its life history, both under experimental conditions and in the field. The aim with the present study is to test whether early sexual development and differentiation in the stickleback can be used as biomarker for endocrine disrupting effects of water-borne steroids. We investigated different time windows of exposure to sex hormones and pulp mill industry effluents to define the most sensitive stage during early development.

There are about 35 000 classified species of crustaceans. To date, no certain case of endocrine disruption has been described in crustaceans, neither in the laboratory nor in the field. Due to the lack of knowledge concerning crustacean endocrinology, much work on endocrine disruption in crustaceans has been carried out with environmental pollutants that have been shown to affect vertebrate endocrine functions. Even though vertebrate-type estrogens have been identified in several crustacean species their functional role has not been established in crustaceans. However, this does not mean that endocrine disruption is not a problem in crustaceans. It rather indicates the need to carry out further, specifically directed research on crustacean endocrinology and potential endocrine disrupters that are specific to crustaceans.

At our institute we have developed a full life-cycle (i.e. 2-generation test) test with the brackish water harpacticoid copepod *Nitocra spinipes* in order to test effects of single substances and complex effluents. The biological endpoints include survival and development of the early life-history stages and aspects of sexual reproduction (e.g. sex ratio, inter-sexuality, time to first egg sac, numbers of offspring produced and population growth). A number synthetic musks, brominated flame retardants (BFR) and effluents from pulp mill industries and dump sites have been tested so far.

Encouraged by our positive experiences with *N. spinipes*, a new draft OECD Test Guideline (TG) has been produced in collaboration with European research colleagues and describes test procedures for measuring the chronic effects of chemicals on initially three species of marine/estuarine copepods: *N. spinipes*, *Tisbe battagliai* and *Acartia tonsa*. The biological attributes of these species facilitate the development from the existing separate test protocols to a common TG for standardized measurement of effects on development, reproduction and life-table analysis. Other potentially useful marine copepod species will also be considered for inclusion in the TG. The remaining work to develop a final and common TG for several copepod species will require international collaboration and agreement. An international *ad hoc* reference group would be a very useful tool in this process.

Assay Validation Studies In Progress in the U.S. Endocrine Disruptor Screening Program

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The U.S. Environmental Protection Agency has several studies currently in the laboratory to assist in the standardization, optimization, and validation of assays for potential inclusion in a chemical screening program for endocrine disruption. These include optimization and validation studies for an estrogen receptor binding assay, a demonstration study for an androgen receptor binding assay, a demonstration of a placental aromatase assay, a standardization study for a steroidogenesis assay, several multi-dose and multi-chemical pubertal male and pubertal female studies, a study evaluating an adult male protocol using several chemicals, an in-utero-through-lactation demonstration study, an exploration of an extension of the first generation in a mammalian two-generation assay, a comparative evaluation of fathead minnow assays, a comparative evaluation of vitellogenin methods in medaka and zebrafish, a multi-chemical evaluation of a short-term reproduction assay using the fathead minnow, an avian 2-generation assay, an avian dosing study, and an avian embryo assay. An amphibian metamorphosis assay based on the prometamorphosis stage is also under development. The status of the assay validation effort in the U.S. will be reviewed briefly. Results of studies completed in the past year will be summarized where possible. A summary of positions taken by the USEPA in the past year on significant scientific issues related to screening for endocrine disruption will also be presented.

U.S. Interagency Evaluation of *In Vitro* Endocrine Disruptor Testing Methods

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A number of manmade and naturally occurring chemicals have been found to alter natural endocrine processes by binding with estrogen and/or androgen receptors and either initiating or inhibiting sex hormone dependent gene activation. Concern over possible adverse health effects of such chemicals led to legislation requiring the U.S. EPA to develop and validate a screening and testing program to identify endocrine disrupting chemicals. *In vitro* estrogen (ER) and androgen (AR) receptor binding and transcriptional activation (TA) assays are proposed as components of a screening battery composed of both *in vivo* and *in vitro* test methods. *In vitro* results will be used as part of a weight-of-evidence decision regarding the need for definitive multigenerational *in vivo* testing to provide data for risk assessments.

Prior to incorporating test methods into the EPA screening and testing program, the methods must be adequately validated and found to be acceptable for their proposed use. The U.S. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), which evaluates new test methods on behalf of federal agencies, was asked to conduct an independent scientific peer review of the validation status of existing *in vitro* ER and AR binding and TA assays. To support the evaluation, the National Toxicology Program Interagency Center for the Evaluation of Alternative Methods (NICEATM) prepared comprehensive reviews of existing protocols and data. An international expert panel was convened in May 2002 to evaluate the available information and to provide conclusions and recommendations about the status and future validation of these assays

The panel concluded that no *in vitro* ER and AR binding or TA assays are sufficiently standardized and adequately validated for regulatory testing. The panel therefore recommended a series of minimum procedural standards that should be incorporated into standardized test method protocols for each of the four assay types, and provided recommendations about the types of assays that should receive priority for future development and validation.

The panel provided recommendations on chemicals that should be used for validation of these assays. To determine the specificity of the assays, they recommended that at least 25 % of the chemicals should be negative for each assay type. Following the panel meeting, NICEATM and an ICCVAM working group compiled a common list of 78 proposed substances that should be considered for validation studies. The list is intended to ensure that assay reliability and performance are adequately characterized for a broad range of chemical classes and across a wide range of potencies from weak to strong. The expert panel and ICCVAM recommendations should facilitate validation and adoption of standardized protocols for ER and AR binding and TA assays that can be used as part of a screening battery to identify potential endocrine disrupting chemicals. The final report of the expert panel's conclusions and recommendations and the background review documents considered by the panel are available at the ICCVAM/NICEATM website: http://iccvam.niehs.nih.gov/methods/endocrine.htm.

Current Strategies against Environmental Endocrine Disrupters by the Ministry of the Environment, Government of Japan

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FOREWORD

Endocrine disrupters have become the focus of great concern among the general public over their adverse effects on human beings and wildlife, but there is still much to be explored scientifically, making Endocrine disrupters a key environmental safety issue. Therefore, the Ministry of the Environment, Japan, is conducting environmental monitoring and hazard assessment, and is also engaged in the exchange of information with other countries and international organizations under a framework of international coordination.

As a part of these activities, the Ministry has held International Symposiums on Environmental Endocrine Disrupters since 1998. The past symposiums brought together leading scientists from all over the world. Their productive discussions were highly appreciated on an international level.

APPROACHES TO ENDOCRINE DISRUPTER ISSUES

In May 1998, MoE developed the "Strategic Programs on Environmental Endocrine Disrupters'98" (SPEED'98) and presented them to the public. The Programs set forth the Ministry's basic perspectives on the problem and specific lines of action on it.

More specifically, SPEED'98 has called for work on the following four items.

(1) Promotion of a fact - finding study on detection in the environment and influence on wild life;

(2) Promotion of testing and research, and technology development;

(3) Assessment and management of environmental risk, and provision of related information; and

(4) Efforts for establishment and reinforcement of international networking on endocrine disrupter issues.

Within 65 chemical substances listed on SPEED'98 as be suspected of having endocrine disrupting effects, 28 chemical substances have been selected and their hazard assessment concerning their risks to human health or ecosystem have accordingly begun. (i.e. 12 chemical substances in 2000, 8 substances in 2001 and 8 substances in 2002)

The results of hazard assessment toward 12 substances selected in 2000 have already been published. Based on them, it was confirmed that two substances, nonylphenol and 4-octylphenol, had endocrine disrupting effects to *medaka* fish.

POLICY FROM NOW ON

MoE is committed to continue efforts to the accumulation of scientific knowledge and hazard assessment etc. to solve this problem by taking into account novel findings such as a WHO-IPCS report entitled "Global Assessment of the State-of-the-Science of Endocrine Disrupters" and approaches in international institutes or organizations.