

**UNITED NATIONS ENVIRONMENT PROGRAMME  
INTERNATIONAL LABOUR ORGANIZATION  
WORLD HEALTH ORGANIZATION**



**REPORT OF THE JOINT IPCS-JAPAN WORKSHOP ON  
“ENDOCRINE DISRUPTORS: RESEARCH NEEDS AND FUTURE  
DIRECTIONS”**

Report Prepared for the WHO/UNEP/ILO International Programme on Chemical Safety.

## **Background**

1. The Ministry of Environment, Japan and the WHO/UNEP/ILO International Programme on Chemical Safety (IPCS) hosted a joint workshop on “Endocrine Disruptors: Research Needs and Future Directions,” in Tokyo, Japan 7-9 December, 2003. A list of participants for the workshop is provided in Annex A. This workshop was convened after the International Symposium on Environmental Endocrine Disruptors 2003, held in Sendai, Japan 3-5 December, 2003.
2. Dr. K. Nogami, Deputy Director, Environmental Health Department, Japanese Ministry of Environment (MOE) welcomed the participants on behalf of the Japanese Ministry of the Environment, and Dr. T. Meredith, on behalf of the World Health Organization (WHO) and the IPCS. Dr. Meredith thanked the MOE, Japan for their financial and logistical support of the meeting. Dr R. Kavlock (US EPA) chaired the meeting and Dr E. Francis (US EPA) served as rapporteur.

## **Objectives of the Workshop**

3. Dr T. Damstra (WHO/IPCS/Interregional Research Unit) stressed that the major focus of the workshop was to follow-up on the conclusions of the WHO/IPCS “Global Assessment of the State-of-the-Science of Endocrine Disruptors<sup>1</sup>” ([http://www.who.int/pcs/pcs\\_new.htm](http://www.who.int/pcs/pcs_new.htm)). This comprehensive assessment of the global scientific literature on EDCs stressed that although scientific research has significantly advanced our understanding of the mechanisms of action, health and ecological effects of EDCs, and extent of exposure to EDCs; numerous uncertainties remain. The document identified critical data gaps and research needs and stressed that because of continuing concerns; studies on the potential effects of these chemicals should remain a high global priority and will require coordinated international efforts.

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<sup>1</sup> Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G (2002) Global Assessment of the State-of-the-Science of Endocrine Disruptors. International Programme on Chemical Safety. WHO/PCS/EDC/02.2. World Health Organization, Geneva, Switzerland.

4. The specific objectives of this workshop were:
  - a) To share the latest scientific findings in specified areas of research on endocrine disrupting chemicals (EDCs).
  - b) To identify the most critical data gaps and research needs that continue to contribute to unresolved EDC issues.
  - c) To discuss future directions and opportunities for future collaborative research initiatives to fill these data gaps.

### **Workshop Agenda**

5. The workshop included presentations [see Annex B] by the participants summarizing the current state-of-the-science of his/her specific research area followed by extensive discussion of key data gaps in that research area by all participants. The group then reached consensus on the top key research needs in that particular scientific discipline.
6. Several presentations [see Annex B] were given by representatives from international, regional, and national organizations on ongoing research programs addressing EDCs, and identifying opportunities for collaborative initiatives among countries and investigators.

### **Summary Of Key Research Needs**

7. The research needs identified during the workshop were integrated and summarized, and aggregated into categories paralleling the chapters in the global assessment document.
8. General
  - a. Conduct focused international meetings that examine specific diseases, target glands, or exposures, to further identify research needs and specific approaches that are required over the next 5 years.

- b. Promote and foster international research programs and international workshops and meetings to facilitate communication, exchange of data, and collaborative research.
  - c. Refine the GAED causal criteria and utilize them to monitor the direction of the “weight of the evidence” over time.
  - d. Develop a database of assessments of putative EDCs conducted by authoritative bodies internationally and nationally.
  - e. Develop up-to-date databases on EDC effects and outcomes to give precise information on the status of current scientific work (e.g., results of screening and testing, comparisons of receptor bindings across species, etc.).
  - f. In conducting studies to evaluate human health and effects in wildlife, address newly emerging endocrine disruptors (e.g., pharmaceuticals, non-persistent and non-bioaccumulative chemicals, fluorinated organics, brominated flame retardants) and other potentially environmental relevant stressors (e.g., hypoxia, nitrates, global warming) as well as non-estrogen, androgen and thyroid dependent pathways (e.g., retinoic acid signaling).
  - g. Research on mixtures and on the role of phytoestrogens and hormonally active pharmaceuticals and their interactions with environmental chemicals is needed to understand the multiple potential health effects of EDCs.
9. *MODES OF ACTION (Chapter 3 in IPCS Global Assessment Document):*
- a. Use genomic approaches to dissect out critical toxicity pathways for use in evaluating dose-response relationships, critical life stages, comparative chemical effects, and cross species extrapolations. The results of this research will improve our ability to understand quantitative relationships between gene expression and higher order effects. This will entail sharing of protocols, approaches, and data interpretation between laboratories.
  - b. Evaluate the utility of concept using different animal models, (e.g., zebra fish) for exploring toxicity pathways for endocrine disruption, while recognizing the potential difficulties of using some species in routine toxicological studies due to differences in basic biological mechanisms.

10. *WILDLIFE EFFECTS (Chapter 4 in IPCS Global Assessment Document):*

- a. Increase our understanding of the basic molecular and biochemical mechanisms underlying endocrine-mediated effects and how these effects translate to responses at the level of the individual, population and ecosystem. Establishment of linkages across biological levels of organization is critical for determining cause and effect relationships. Further research on diverse species is required to improve our ability to extrapolate EDC-induced effects across species.
- b. Increase the long term monitoring of sentinel populations to evaluate temporal trends and effectiveness of remediation efforts.
- c. Evaluate the appropriateness of using laboratory/field data in population modeling of the effects of EDCs. This will require understanding baseline data and evaluation of population sustainability, recruitment, fecundity, and survival.
- d. Conduct further laboratory and field studies to investigate the linkage between EDC-induced endocrine dysfunction and immunotoxicity, particularly in marine mammals.

11. *HUMAN HEALTH EFFECTS (Chapter 5 of IPCS Global Assessment Document):*

## Developmental/Reproductive Effects

- a. Develop a coordinated research program using experimental models to assess the role of EDC exposures in causing congenital alterations in male reproductive tract development (e.g., altered anogenital distance, hypospadias, cryptorchidism) using modern molecular techniques (e.g., -omics).
- b. Promote international coordination (including developing countries) of longitudinal studies of children's health, using harmonized protocols.

## Neurological/Neurodevelopmental and Thyroid Effects

- c. Clarify the impact of EDCs (including newly emerging EDCs such as PFOS and PBDEs) on thyroid hormone homeostasis and on neurodevelopmental effects. Conduct epidemiological and

experimental studies that address interactions with marginal iodine deficiency and subclinical hypothyroidism.

- d. Develop peripheral thyroid-specific markers of response that can be used to diagnose alterations in thyroid hormone functions. These markers should be evaluated at critical life stages and may be incorporated into a mammalian two-generation reproductive study.
- e. Characterize the impact of PAHs and newly emerging EDCs on hormonal systems involved in the determination of sex-role identity and/or gender-specific cognitive functions. Special attention should be given to exposures during brain development.
- f. Further clarify the role of socioeconomic factors (using existing cohorts) in influencing the persistence of adverse neurodevelopmental outcomes resulting from exposures to PCBs, DDT, metals, etc.

#### Immune Effects

- g. Expand studies of interrelationships between the immune and endocrine systems to better understand the potential effects of EDCs on the immune system. Priority studies may include epidemiological studies of highly exposed children in developing countries (taking advantage of immunization effectiveness records).

#### Cancer (and other chronic diseases)

- h. Develop predictive indicators and determine individual genetic risk factors that might contribute to incremental risk of induction of endocrinopathies, (e.g. cancer and cardiovascular diseases).
- i. Develop biomarkers of cumulative exposure to EDCs to understand the potential effects of EDCs on human cancer incidence.
- j. Determine the critical period during which exposure to EDCs, particularly in earlier life stages, may be related to the induction of endocrine-mediated cancers (especially for breast cancer).

- k. Develop standardized molecular epidemiological protocols (e.g., nested case control prospective studies) that could be used to study potential EDC-related causes of cancer for use in developing countries.

12. *EXPOSURE: ASSESSMENT AND MONITORING OF EDCs (Chapter 6 in IPCS Global Assessment Document):*

Research on exposure to mixtures underlies all exposure assessment and monitoring needs.

Methods

- a. Develop and validate bioassay systems for specific modes/mechanisms of action for monitoring complex mixtures to serve as a basis for biologically-based fractionation studies to identify chemicals responsible for adverse effects.
- b. Develop highly sensitive and selective chemical and biological analytical methods for EDCs and their metabolites, including novel technologies such as biosensors and nanotechnology. These methods need to have high through-put capabilities, and be cost effective.
- c. Develop methodologies to address the impact of EDC exposures on developing organisms and whether or not there is a delayed onset of the EDC-induced effect. In order to understand whether adult onset of disease may have a fetal or childhood origin, it is also important to assess exposures that took place 20-30 years ago.
- d. Develop best management practices to reduce the risks associated with the use and release of EDCs of concern. This may involve the development of methods and techniques for treating wastes (municipal, industrial, and agricultural) and develop best management practices for intensive agriculture, particularly in the use of biosolids.

Models

- e. Validate models to predict loading, fate and exposure to EDCs including global redistribution, bioaccumulation, and pharmacology (i.e., target sites) of both PBTs and chemicals of interest that cause continuous exposure. Improve the understanding of biological transformation and geochemical cycling of chemicals in biota and the environment.

## Measures

- f. Include measurement of the internal dose of chemicals in both field and laboratory studies aimed at human health and wildlife effects assessments.
- g. Identify and characterize exposures of susceptible human and wildlife subpopulations to EDCs, including critical temporal and spatial aspects relative to sensitive life stages. Identify and characterize EDC exposure of humans and wildlife subpopulations that have potential high exposures due to life history (e.g., occupation, diet, trophic status, other factors - e.g., agriculture, top predators, arctic mammals).
- h. Identify priority chemicals to be included in broad spectrum surveys of EDCs in various environmental media. Initiate harmonized and carefully designed monitoring programs that allow comparisons of the data overtime and across jurisdictions, and geographic regions.
- i. Develop better methodologies (e.g., validation of dietary surveys) for estimating human exposure to phytoestrogens.

13. *METHODOLOGIES FOR ASSESSING EDCS (Addressed by OECD; not in IPCS Global Assessment Document)*

- a. Improved methodologies (e.g., high-throughput screening) to prioritize chemicals for subsequent selection in more extensive screening/testing methodologies and strategies are urgently needed.
- b. Improve basic knowledge of the biology of species used in screening and testing programs, so that there are benchmarks for evaluating the normal variability of endpoints measured compared to a significant response/effect after chemical exposure and for building stronger foundation of extrapolation across species. Research is especially needed on crustaceans, annelids and mollusks, to help fill current data gaps in biology of invertebrates.
- c. Identify endpoints in the amphibian metamorphosis assay indicative of mode of action that might be used to extrapolate findings to other species. This will necessitate the parallel assessment of chemicals in the various screening tests for thyroid effects.



- d. Standardize emerging technologies (e.g., biomarker protein measurement methods) for screening purposes.
- e. Receptor binding and TA assays exist but have not been standardized or validated. Specificity, particularly for androgen TA assays is still not adequate, and better test methods (particularly those that do not detect glucocorticoids as positives) are needed.
- f. Develop mode-of-action specific gene expression patterns (whole genome transcript profiling using cost-effective methods) for various endocrine modalities (e.g., in the developing rodent) for the purpose of establishing dose, time and tissue responses for established toxicological and pharmacologic probes. This will assist in establishing a database of toxicity pathways that can be used to define adverse profiles of gene expression and to develop predictive models of toxicity.
- g. Develop less expensive and labor-intensive alternative methods that can be used to bridge between screening (Tier 1) and testing (Tier 2) assays, (e.g., to assess the toxicity of chemicals that have a lower potential for human exposure).
- h. Develop a better understanding of the predictivity of newly developed methods. QSAR approaches may help prioritize certain classes of chemicals, but may be limited in determining testing strategies due to the interactive and multiple mechanisms of action of EDCs.
- i. Conduct and compare results for multigenerational and full- or partial-life cycle test designs for assessing the effects of EDCs in fish.
- j. Conduct a comprehensive analysis of results from standard developmental and reproductive toxicity tests in rodents to determine the degree of sensitivity of apical end points to detect EDCs
- k. Conduct a coordinated, validated research program to assess whether certain chemicals (e.g. bisphenol A) cause effects at “low doses”.

## **Conclusions**

The workshop achieved its objectives and provided an excellent opportunity to a) discuss the latest scientific findings on specific EDC-related research areas; b) prioritize research needs in those areas;

and c) identify opportunities for international collaboration. The participants acknowledged that an abundance of data gaps and research needs were identified, and that they needed to be further focused and refined. It was recommended that a follow-up workshop focus on a specific scientific area, and address in greater detail the critical data gaps that, if filled, hold the potential of advancing the science and resolving uncertainties.

ANNEX A

JOINT IPCS – JAPAN WORKSHOP ON  
“ENDOCRINE DISRUPTORS: RESEARCH NEEDS AND FUTURE DIRECTIONS”

7-9 DECEMBER 2003  
TOKYO, JAPAN

List of Participants

*Members*

Dr Gerald Ankley, Mid-Continent Ecology Division, US Environmental Protection Agency, National Health Effects Environmental Research Laboratories, 620 Congdon Boulevard, Duluth, Minnesota 55804, USA, Tel: 218-529-5147, Fax: 218-529-5003, Email: [ankley.gerald@epa.gov](mailto:ankley.gerald@epa.gov)

Dr Mariano E. Cebrian, Centro de Investigacion Y De Estudios Avanzados Del I.P.N., Departamento de Farmacologia Y Toxicologia, Av. Instituto Politecnico Nacional 2508, Col. San Pedro Zacatenco, Mexico, D.F. C.P. 07300, Tel: 5747-3800 ext. 5433; Fax: 5747-7095; Email: [mcebrian@mail.cinvestav.mx](mailto:mcebrian@mail.cinvestav.mx)

Dr George Daston, Miami Valley Laboratories, Procter & Gamble Co., P.O. Box 538707, 11810 E. Miami River Road, Cincinnati, Ohio 45253, USA, Tel: 513-627-2886, Fax: 513-627-0323, Email: [daston.gp@pg.com](mailto:daston.gp@pg.com)

Dr Elaine Francis, Endocrine Disruptors Research Program/ORD, US Environmental Protection Agency, Ariel Rios Bldg. – (8701R), 2200 Pennsylvania Ave., NW, Washington, D.C. 20460, USA, Tel: 202-564-6789, Fax: 202-565-2444, Email: [francis.Elaine@epa.gov](mailto:francis.Elaine@epa.gov)

Dr Anne Gourmelon, OECD Environment, Health & Safety Division, 2, rue Andr J-Pascal, 75775 Paris Cedex 16, France, Tel: 33-1-4524-9849, Fax: 33-1-4524-1675; Email: [anne.gourmelon@oecd.org](mailto:anne.gourmelon@oecd.org)

Dr Jerry Heindel, Division of Extramural Research and Training, National Institute of Environmental Health Sciences, P.O. Box 12233, Mail Drop EC/3413, Research Triangle Park, North Carolina 27709, USA, Tel: 919-541-0781; Fax: 919-541-5064; Email: [heindelj@niehs.nih.gov](mailto:heindelj@niehs.nih.gov)

Dr Taisen Iguchi, Okazaki National Research Institutes, Center for Integrative Bioscience, 5-1 Higashiyama, Myodaiji, Okazaki 444-8585, Japan, Tel: 81-564-59-5235, Fax: 81-564-59-5236, Email: [taisen@nibb.ac.jp](mailto:taisen@nibb.ac.jp)

Dr Tohru Inoue, Cellular and Molecular Toxicology Division, Biological Safety Research Center, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Tel: 81-3-3700-9619, Fax: 81-3-3700-9647, Email: [tohru@nihs.go.jp](mailto:tohru@nihs.go.jp)

Dr Jun Kanno, Division of Cellular & Molecular Toxicology, National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan, Tel: 81-3-3700-9619; Fax: 81-3-3700-9647; Email: [kanno@nihs.go.jp](mailto:kanno@nihs.go.jp)

Dr Robert Kavlock, US Environmental Protection Agency, Reproductive Toxicology Facility, Mail Drop – 71, Research Triangle Park, NC 27711, USA, Tel: 919-541-2326, Fax: 919-541-1499, Email: [kavlock.robert@epa.gov](mailto:kavlock.robert@epa.gov)

Dr Lizbeth Lopez-Carrillo, Mexico Institute of Public Health, Av. Universidad No. 655, Col. Sta. Ma. Ahuacatitlan C.P. 62508, Cuernavaca, Morelos, Mexico, Tel: 52-777-3-29-3000, Fax: 52-777-3-1123-38, Email: [lizbeth@correo.insp.mx](mailto:lizbeth@correo.insp.mx)

Masatoshi Morita, National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki, 305-8506, Japan, Tel: 81-29-850-2332, Fax: 81-29-850-2570, Email: [mmorita@nies.go.jp](mailto:mmorita@nies.go.jp)

Dr Ragnor Pedersen, Representative for EU, Centre for Toxicology, University of London School of Pharmacy, 29/39 Brunswick Square, Bloomsbury, London WC1N 1AX, United Kingdom, Tel & Fax: 44-20-7753-5811; Email: [ragnor.Pedersen@ulsop.ac.uk](mailto:ragnor.Pedersen@ulsop.ac.uk)

Dr Mark Servos, Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario N2L3G1, Canada, Tel: 519-888-4567 ext. 6034, Fax: 519-883-7574; Email: [mservos@uwaterloo.ca](mailto:mservos@uwaterloo.ca)

Dr Glen Van Der Kraak, University of Guelph, Department of Zoology, 80 Stone Road, East Guelph, Ontario N1E 4H5, Canada, Tel: 519-824-4120 ext 2593, Fax: 519-767-1656, Email: [gvanderk@uoguelph.ca](mailto:gvanderk@uoguelph.ca)

Dr Jeff Vos, RIVM (National Institute of Public Health), A. van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands, Tel: 31-30-274-2075, Fax: 31-30-274-2971, Email: [j.vos@rivm.nl](mailto:j.vos@rivm.nl)

Dr Gerhard Winneke, University of Dusseldorf, Medical Institute of Environmental Hygiene, Auf'm Hennekamp 50, D-4000 Dusseldorf 1, Germany, Tel: 49-211-3389-291, Fax: 49-211-3190-910, Email: [Gerhard.winneke@uni-duesseldorf.de](mailto:Gerhard.winneke@uni-duesseldorf.de)

Dr Yoshimasa Shishiba, KKR Mishuku Hospital, 5-33-12, Kami-meguro, Meguro-ku, Tokyo 153-0051, Japan, Tel: 81-3-3711-5771; Fax: 81-3-3792-1682; Email: [shishibayos@aol.com](mailto:shishibayos@aol.com)

*Observers*

Dr Kazuhiko Adachi, Director of Environmental Health and Safety Division, Environmental Health Department, Japanese Ministry of the Environment, Tokyo, Japan, Tel: 81-3-5521-8261, Fax: 81-3-3580-3596, Email: [Kazuhiko\\_adachi@env.go.jp](mailto:Kazuhiko_adachi@env.go.jp)

Dr Koujirou Nogami, Environmental Health and Safety Division, Environmental Health Department, Japanese Ministry of the Environment, Tokyo, Japan, Tel: 81-3-5521-8261, Fax: 81-3-3580-3596, Email: [koujirou\\_nogami@env.go.jp](mailto:koujirou_nogami@env.go.jp)

Dr Hiroyuki Hori, Environmental Health and Safety Division, Environmental Health Department, Japanese Ministry of the Environment, Tokyo, Japan, Tel: 81-3-5521-8261, Fax: 81-3-3580-3596, Email: [hiroyuki\\_hori@env.go.jp](mailto:hiroyuki_hori@env.go.jp)

Mr Yashiro Tanabe, Environmental Health and Safety Division, Environmental Health Department, Japanese Ministry of the Environment, Tokyo, Japan, Tel: 81-3-5521-8261, Fax: 81-3-3580-3596, Email: [yashiro\\_tanabe@env.go.jp](mailto:yashiro_tanabe@env.go.jp)

*WHO Secretariat*

Dr Terri Damstra, World Health Organization, International Programme on Chemical Safety, PO Box 12233, MD EC-33, Research Triangle Park, North Carolina 27709, USA, Tel: 919-541-7537, Fax: 919-541-3276, Email: [Damstra@niehs.nih.gov](mailto:Damstra@niehs.nih.gov)

Dr Tim Meredith, World Health Organization, International Programme on Chemical Safety, 20, Avenue Appia, 1211 Geneva 27, Switzerland, Tel: 41-22-791-3590, Fax: 41-22-791-4848, Email: [mereditht@who.int](mailto:mereditht@who.int)

ANNEX B**TITLE: Needs of Toxicogenomics, Ecotoxicogenomics and Proteomics for Understanding Mode of Action of Chemicals on Humans and Wildlife**

**AUTHOR:** Taisen Iguchi, Center for Integrative Bioscience, Okazaki National Research Institutes, Myodaiji, Okazaki, Japan, [taisen@nibb.ac.jp](mailto:taisen@nibb.ac.jp)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

We applied a differential display method to detect genes related to ovary-independent vaginal changes induced by estrogens including diethylstilbestrol (DES) during the critical developmental windows in mice, and cloned a serine proteinase, neuropsin, and a new C-type lectin, which were expressed only in the vagina exhibiting ovary-independent changes induced by neonatal DES-exposure (1,2). We also found that the persistent expression of EGF-family growth factors and phosphorylations of erbBs receptors and estrogen receptor  $\alpha$  in the neonatally DES-exposed mouse vagina (3). A cDNA microarray method was used to detect estrogen-responsive genes in the mouse reproductive tracts stimulated by estrogenic chemicals including 17 $\beta$ -estradiol ( $E_2$ ), DES, nonylphenol (NP) and bisphenol-A (BPA). The method was successfully applied to genome-wide analysis of gene expression stimulated by hormones and/or chemicals, and also applicable to toxic effect of chemicals. We demonstrated that expressions of a large number of genes were affected by estrogen, and genes that showed higher reproducibility in results repeated experiments were selected. For most of the selected genes, expression was induced in a dose-dependent manner. Gene expression was not altered following  $E_2$  treatment in estrogen receptor  $\alpha$  knockout ( $\alpha$ ERKO) mice. Multiple genes related to sterol biosynthesis, tRNA synthesis, RNA processing, and growth signaling were activated. Activation of these genes suggests a basis for the marked uterotrophic effect observed several days following estrogen administration. Characteristic gene expression patterns were observed for each estrogenic chemical, and these were distinct from that for  $E_2$ , suggesting specific mechanisms of action for endocrine disruption that could be different from that induced by endogenous estrogen (4-6). cDNA microarray was successfully applied to understand gene expression in the testis of neonatally DES exposed mice and found that genes showing altered expression in testis of adult mice following prenatal DES exposure (7-9).

We have applied cDNA microarray for understanding molecular basis of adverse effect of chemicals such as benzo(a)pyrene, NP, BPA, cadmium chloride, testosterone and  $E_2$  on growth of *Caenorhabditis elegans* (10,11).

Daphnids have been widely used for aquatic toxicology. We have found that styrene dimers and styrene trimers leached out from disposable polystyrene cups reduced number of offspring of *Ceriodaphnia dubia* at very low concentrations (12). Juvenile hormone agonists reduced the number of offspring and induced male offspring not only in *Daphnia magna*, but also in *C. dubia* and *Monia macrocopa*, and these chemical acted only during early oogenesis (13). We are currently establishing a cDNA microarray of *D. magna*.

In all species of crocodilians, sex is determined not by genetic mechanisms alone, but also by the temperature at which the egg is incubated. In the American alligator (*Alligator mississippiensis*), the thermosensitive period (TSP) for sex determination is the 7- to 10-day window within stages 21-24 of development. Treating embryos with estrogen during the TSP produces female

offspring, even at male incubation temperatures. Therefore, estrogens play a role in determining sex in the alligator. However, the mechanisms of estrogen action on sex determination in the alligator are still uncertain. Studies of contaminant-exposed alligators showed alterations in steroid action. Whether these abnormalities are due, in part, to alterations in steroid receptor expression remains unclarified. To understand action mechanisms of steroids and chemicals in alligators, we isolated cDNA encoding the ERs and the progesterone receptors (14), androgen receptor (AR), aromatase etc. We are establishing a cDNA microarray of the American alligator.

In order to establish a model system for studying the effects of environmental chemicals on marine fish, we cloned ERs and established reporter gene assay system, and examined the effects of E<sub>2</sub> on early development of *Fundulus heteroclitus*. Alkylphenols had high binding affinity to *Fundulus* ER $\alpha$  and higher affinity than human ER $\alpha$  to alkylphenols (15). We also cloned ARs from mosquito fish and analyzed developmental effect of trenbolone on the gonopodium.

To understand the molecular mechanisms of estrogenic effects on developing *Xenopus laevis* embryos, we used a NIBB *Xenopus* cDNA microarray. We observed malformations and altered gene expression in *X. laevis* embryos exposed to high concentrations of BPA, NP and E<sub>2</sub> (Sone *et al.*, unpublished data). We are studying genes expressed differentially during transformation of the ovary into testis in *Rana rugosa* tadpoles exposed to testosterone propionate.

Microarray is a powerful tool for identifying changes in global gene expression during development and further help us understanding the molecular basis of adverse effect of sex hormones and environmental hormones that can alter signaling systems. We need to know more basic biology in each animal species.

#### TOP FIVE KEY RESEARCH RECOMMENDATIONS:

1. In order to clarify the mode of action of chemicals, understandings of receptor systems, such as ERs, AR, PR, AhR, aromatase etc. in each animal species, and cross-talk of receptors, and cofactors related to receptor systems are essential.
2. We need to know normal development and sensitive windows during development (critical period) in each animal species.
3. Understanding the Genome in several species should be completed in the near future, which will help understanding of global gene expression in each animal species.
4. Understanding metabolism of chemicals and nuclear transcription factors are essential to clarify the mode of action of chemicals.
5. We need to know what is normal and what is abnormal in each species. Especially sex determination, developmental and differentiation pattern of gonads should be clarified in each animal species.

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**TITLE:**

**AUTHOR:** Tohru Inoue, Cellular and Molecular Toxicology Division, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan, [tohru@nihs.go.jp](mailto:tohru@nihs.go.jp)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

1. Following introductory notes on on-going endocrine disruptor research projects are presented by Japanese member:
  - 1) For environmental surveillance on the effect of wild life, Ministry of Health, Labor and Welfare (MHLW) has been continuously evaluating the exposure dose of human life to selected environmental chemicals, and assessing their possible epidemiological impact for particular diseases; Ministry of Agriculture, Fishery, and Forestry (MAFF) surveys possible impact on the agricultural and oceanic ecosystems including the yield of crops and the haul of fishery; Ministry of Road Construction and Transportation (MRCT) focuses the environmental surveillance in swages, oceanic and inland water, etc. Technical development of analytical chemistry for EDCs is conducted by MRCT; Ministry of Environment (MOE) is surveying environmental pollutants and their impact on the ecosystem and assessing environmental exposure due to possible EDCs listed previously. The MOE has been trying to renovate and develop a new one generation study to cover a possible data gaps during foetal and new born exposure.
  - 2) For developing new testing and conducting mechanistic studies, Ministry of Education, Culture, Sports, Science and Technology (MECSST) strongly promotes a basic science and research on endocrine disruptors, dioxins, and POPs-related chemicals. Furthermore, MECSST plans to develop safety science and technologies, and to provide current information on environmental risk-assessment and management; The MHLW and METI are cooperatively proceeding and validating a series of methods for screening and testing in *in vitro* as well as *in vivo*, covering various levels such as *in silico*, *in vitro*, *ex vivo* and *in vivo* assays including validation of the uterotrophic assays. The METI also focuses on the QSAR system for prescreening; MAFF evaluates a variety of screening methods for pesticides, possible exposure of pesticides to human life, the ecosystem, and the oceanic wild life. MAFF focuses on the effects of environmental pollutants on agriculture and fishery, as well.
  - 3) Environmental risk-assessment and risk-management concern, MECSST focuses on the education system of environmental protection from pollutant for school children, and releases information through an Internet; MHLW strengthen the international cooperative network on exchanging information of endocrine disruptors. Together with METI, MHLW assays *in vitro* screening system using a robotic system, and also trying to establish new testing methodologies.

2. Possible global collaboration and recommendations.

- 1) Continuous international research collaborations are required in a particular area, such as children's issue, to which fund is limited but available from MECSST.
- 2) Fund for International research collaboration: Limited but funding scheme is available from MECSST (Applying the global research collaboration to MECSST from WHO/IPCS).
- 3) Recommendation of global collaborations by steering committee in particular research area; Simultaneous voluntary studies for possible global collaborations are recommended.
- 4) Recommendation of experimental guideline for EDC-research. cf. Use of "phytoestrogen-free dietary standard" established by J. Kanno, NIHS, Japan.
- 5) In order to encourage a global research, an international workshop on each particular topic is recommended.

3. Policy development of the EDC research and recommendation.

- 1) Encouraging research on the scientific fundamental key-issues in the EDC-research. cf. sex reversal genes, etc.
- 2) Encouraging research on common subjects in EDC-research risk identification and better understanding. cf. special focusing on BPA (across species, strains and endpoints).
- 3) EDC-researches, encouraging local community to get rid of unhealthy traditional habit(s); encouraging to improve children's and their mothers' health. cf. potential hazard of fish products in particular area, polluted with PBB, PCB, etc.
- 4) Recommendation of research on the early onset of pubertal stage as a consequence of effects of EDCs and biological relevancy.
- 5) Synergy and additive issue in EDCs: Encouraging workshop on the issue of synergy and additive.  
Mechanistic study on synergy of EDCs with As and Zn.  
Stem cells & Effect of EDCs

**TITLE: Endocrine Disruptors and Human Health**

**AUTHOR:** Jerrold J. Heindel, Division of Extramural Research and Training, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, North Carolina, USA, [heindelj@niehs.nih.gov](mailto:heindelj@niehs.nih.gov)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

In the year or so since the WHO Global Assessment of the State-of-the-Science of Endocrine Disruptors the statement that there is no direct cause and effect relationship between exposure to endocrine disrupting chemicals and any human reproductive disease or dysfunction remains true. As stated in the original document, there is biological plausibility for an effect of endocrine disrupting chemicals on such reproductive parameters as puberty, polycystic ovary syndrome, premature menopause, endometriosis, uterine fibroids, reduced sperm counts, time to pregnancy, and fertility as well as developmental endpoints of cryptorchidism and hypospadias. However the data are still inadequate to support the conclusion that endocrine disrupting chemicals adversely affect human reproductive health. The main problems still lie in the lack of adequate exposure data and our ability to determine that the adverse effects detected are due to disruption of the endocrine system.

The major issues concerning the significance of exposure to endocrine disrupting chemicals in human reproductive health are related to improved exposure assessment and our ability to relate exposures to adverse effects and the adverse effects to endocrine disrupting mechanisms. Since human exposures are expected to be at low levels of environmental chemicals, the low dose concept needs to be validated in animals in order to show it is possible for human effects at these low doses. In this regard, there are now data in mice ( B6C57B, ICR, CD1 and CF1) that low doses of bisphenol A ( BPA) in utero, e.g. doses of less than 100ug/kg/d, can cause aneuploidy in female germ cells, changes in mammary gland development, alterations in behavior, early onset of puberty, decreased daily sperm production and altered prostate weight and gland development. Some of the discrepancies in the BPA data relate to the use of the Sprague Dawley rat model, tissue specific effects and the effect of diets. It appears that while the developing nervous system SD of rat is sensitive to the low dose effects this strain of rats is not as sensitive to the reproductive effects of BPA as other rat strains. Indeed, it appears that Bisphenol A is not a reproductive toxicant in the SD rat. However, in utero exposure to low doses of BPA in this and other rat strains does cause effects on estrous cyclicity, aggressive behavior and the size of the locus coeruleus indicating that the developing nervous system is sensitive to low doses. There are also data showing that the uterus is not sensitive to BPA in either rats or mice. This might indicate that BPA is actually a selective endocrine receptor modulator (SERM) and not a general endocrine disrupting chemical. Recent data also indicates that BPA binds to the thyroid hormone receptor that could be responsible for some of the confusing data. However it is not clear if the low dose effects are applicable to all endocrine disrupting chemicals or if they are specific to BPA since it is not a typical endocrine disruptor. It is also clear that diet can affect the BPA effect depending on the content of phytoestrogens and other estrogenic components. Therefore when examining low dose effects one must keep in mind there may be chemical specific effects, tissue specific effects, strain and species differences, dietary and caging effects as well as timing of exposure effects. Thus the low dose effects of BPA are becoming clearer but more data are needed. Once this data is obtained, it will certainly aid the plausibility of EDC having effects in humans who for the most part are only exposed to these low environmentally relevant concentrations.

In examining the effects of EDC in humans one must be cognizant of the following information which contributes to the difficulty in proving the EDC hypothesis in humans: the effects will probably be small, they will be difficult to detect due to human genomic variability and genetic polymorphisms in the population, exposure is likely to be to multiple chemicals with various half lives and differing sensitivities, and in utero exposure will probably be the most sensitive window of exposure and thus result in the most damaging effects on human health. One of the major problems hindering development of a cause and effect relationship between an exposure to EDC and subsequent dysfunction or disease is the long latent period between exposure(s) and the toxicity. Thus the ability to generate the needed data to prove a cause and effect relationship between exposure to an EDC and a subsequent morphological or functional change will be aided by a closer proximity between the exposure and the toxicity. Therefore it seems logical to focus attention on the role of EDC in the developmental basis of disease. The developmental basis hypothesis has the following tenets: The initial exposure can be in utero and/or neonatal; sensitivity to the environmental agents will vary according to the environmental chemical, stage of development, dose, timing of exposure, gender, tissue and genetic makeup; there can be additional exposures to the same or different chemicals throughout life that may further increase susceptibility; the environmental insult may be additive or synergistic with nutritional influences; the resulting pathology can have a latent period from days to years to decades; and finally the altered programming leading to the increased susceptibility to disease/dysfunction can be measured using tools of genomics and epigenetics.

The general approach to the developmental basis of disease and dysfunction due to exposure to environmental chemicals involves developmental exposure (in utero or neonatal depending on the tissue/organ examined) and showing that this exposure results in irreversible changes in the tissue e.g altered cell number or gene expression changes. This is followed by assessment of some disease/dysfunction later in life with measurement of gene expression in the diseased tissue and correlation of the irreversible changes during development to the changes in diseased tissue. Finally, it will be critical to show cause and effect relationship between altered gene expression (for example) and the disease/dysfunction and the mechanism responsible for the altered gene expression. The recent publications by Mori et al have provided evidence that indeed one can measure toxicant levels in humans during pregnancy including fetal blood levels and that these changes can be correlated with altered gene expression in fetal tissue e.g. fetal cord. Therefore, the methods and procedures exist to approach the fetal basis hypothesis in humans.

The following scenario provides a logical approach to detecting endocrine disruptor effects in humans to determine that the effects can be related to endocrine disruption. First, there must be a sensitive target that can be measured noninvasively using quantifiable and standardized methodology. There must also be a sensitive standardized analytical measurement of exposure; the exposure must be relatable to the effect; there must be an animal model with solid data and a mechanism proposed in the animal model that extrapolates to humans, involves the endocrine system and is active at doses relevant to humans. Thus while it is important to determine the effect of EDC on breast/prostate cancer, ovarian/testicular cancers, puberty, fertility, semen quality, hormone levels, menstrual cycle abnormalities, PCOS, endometriosis, aneuploidy, hypospadias (non syndromic), cryptorchidism and anogenital distance to name a few, it is important to choose an endpoint that meets the above criteria. The endpoints that seem the best fit include hypospadias, cryptorchidism and anogenital distance. With regard to hypospadias, for example, it is an endocrine sensitive target, it is an adverse effect, the endpoint can be measured in close proximity to exposure, measurement is noninvasive and quantifiable, there are strong animal data on the mechanism and timing of the effect, and the animal data show effects at environmentally relevant exposures. Thus using the approach of Dr Mori to measure exposure

and gene expression along with measurement of hypospadias, cryptorchidism and anogenital distance at birth could be a viable scenario to provide the needed data to show a cause and effect relationship between exposures to an EDC and a toxic endpoint or disease/dysfunction.

The effect of endocrine disrupting chemicals on human sperm counts is still a controversial area due to problems in quantitation of sperm counts and exposures and the inability to link the exposures to the reduced sperm counts. It seems clear that there is not a general worldwide decline in sperm counts. However it seems increasingly possible that there are regional differences that may be related to specific exposures. Since sperm are constantly being renewed, it should be possible to assess the effects of endocrine disruptors not only during development but also in the adult. More emphasis should be put into determining the effects of acute or subchronic exposures to pesticides with endocrine activity over time in highly exposed populations as it should be possible to link these exposures to the sperm counts.

#### **TOP FIVE KEY RESEARCH RECOMMENDATIONS:**

1. Determine the validity of the low dose phenomenon in animal models including determination if the effect is specific to bisphenol A.
2. Focus research on the developmental basis of disease/dysfunction as the most promising approach to determining cause and effect relationship for EDC on human health.
3. Improve and expand the use of fetal measurements of exposure including use of meconium and their correlation with gene expression in cord tissue.
4. Develop better biomarker of disease/dysfunction using the tools of genomics, proteomics and metabolomics as well as develop a SNP database to aid in understanding differences in susceptibility.
5. Improve interactions and collaborations between investigators developing animal models of disease/dysfunction and those studying human populations.

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**TITLE: Critical Data Gaps/Research Needs: Immune System**

**AUTHOR:** J.G Vos, National Institute of Public Health, Bilthoven, The Netherlands,  
[j.vos@rivm.nl](mailto:j.vos@rivm.nl)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

To determine causality between a chemical contaminant and health effects in a wildlife species is not possible on a statistical association between a biological effect and the contaminant in question as most persistent pollutants covary and thus it is not possible to state equivocally that the biomarker response has been caused by a particular contaminant. In addition there may be other contaminants not analyzed that are just as important. This means that for most reported biological effects in wildlife the evidence for a causal link with a specific chemical contaminant is weak or non-existing. This is mainly due to the complexity of contaminant mixtures, the lack of chemical exposure data, of data on the sensitivity of the species concerned, and of knowledge on mechanisms of action. Understanding the linkages between contaminants and health effects of concern (*e.g.* reproductive disorders or immunosuppression), is most likely to come from studies in laboratory animals. These surrogate species are particularly useful in studies of mechanisms of action and for the determination of dose-response relationships. Crucial in establishing causal evidence for chemical-induced wildlife effects are, therefore, semi-field or laboratory studies using the wildlife species of concern. Semi-field studies represent a useful approach to bridge the gap between the controlled conditions of the laboratory experiments and the uncontrolled exposure conditions in the field. On the basis of these different types of studies a weight-of-evidence argument can be established.

In 1988, approximately 20,000 harbour seals (*Phoca vitulina*), representing up to 60% of local populations died in north-western Europe due to an outbreak of phocine (seal) distemper virus, a previously unidentified morbillivirus. To evaluate whether contaminants at ambient environmental levels can affect immune function of seals, a semi-field immunotoxicological study was carried out, in which captive harbor seals were fed herring from either relatively uncontaminated sites of the Atlantic Ocean, or from the highly contaminated Baltic Sea. Changes in immune function were monitored over a 2<sup>1</sup>/<sub>2</sub>-year period. Seals from the Baltic group had diminished T-cell function *in vitro* and *in vivo*, and reduced natural killer cell function, both of which are crucial to anti-virus defences in vertebrates. Additional (perinatal) feeding studies in rats using the identical herring diets, together with a positive control group exposed to dioxin, confirmed that the “dioxin-like” PCBs were largely responsible for the effects observed and confirmed that perinatal exposure represents a greater immunotoxic threat than exposure as a juvenile or adult (Ross et al., 1996). These data indicate that current concentrations of PCBs in the aquatic food chain in north-western Europe are immunotoxic to marine mammals. This may result in diminished host resistance and an increased incidence and severity of infectious disease (Ross et al., 2000; Vos et al., 2003). The several distemper virus-associated mass mortalities observed since 1987 have been largely restricted to the more contaminated marine mammal populations (Harvell et al., 1999; Ross et al., 2003). A new outbreak of the seal distemper virus in 2002 killed again in north-western Europe 22,000 harbor seals.

Recently, negative associations were found in polar bears from Svalbard and Churchill, Canada between PCBs and serum IgG levels and between PCBs and increased antibody titres against influenza- and reo virus following immunization. These findings may indicate that even in remote areas high levels of organochlorines may impair the polar bears ability to produce antibodies and thus may impair the resistance to infectious agents (Lie et al., In press).

Both human and wildlife toxicology face the challenge of identifying specific chemicals responsible for toxic effects in cases of complex exposures. Ultimately, it is the “weight of evidence” from laboratory, semi-field and field/epidemiological studies that highlighted PCBs as a significant immunotoxic threat to the health of not only the more highly exposed wildlife species but also humans, as background perinatal PCB/dioxin exposure has been shown to influence immune parameters in Dutch children (Weisglas-Kuperus et al., 2000; ten Tusscher et al., 2003). The valuable lessons learned from the more highly exposed wildlife species compel us to better integrate human and ecological research to assess the continued health risks of persistent organohalogen pollutants.

#### TOP FIVE KEY RESEARCH RECOMMENDATIONS:

- 1) Conduct further field and semi-field studies to establish cause-and-effect relationships regarding immunotoxicity in wildlife species, notably in marine mammals that are at the top of the food chain.
- 2) Further evaluate the human immune effects which have been associated with endocrine disrupters and to identify the underlying causes, with special attention to exceptional high chemical exposures and to children.
- 3) Expand developmental immunotoxicity studies from the relatively well documented information on immunosuppression to allergy and autoimmunity.
- 4) Expand studies of interrelationships between the immune and endocrine systems to better understand the potential effects of EDCs on the immune system.
- 5) Amend the current OECD guidelines for reproductive toxicity testing so that the developing immune system is considered as a potential target of toxicity.

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**TITLE: Effects of endocrine disruptors on neural development through thyroid hormone axis**

**AUTHOR:** Yoshimasa Shishiba, Science Council of Japan, KKR Mishuku Hospital, Tokyo, Japan, [shishibayos@aol.com](mailto:shishibayos@aol.com)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

An endocrine system essential for brain development and for which there is growing evidence that environmental chemicals may be interfering with its action is that of thyroid hormone (TH). As early as the first trimester of pregnancy, fetal brain tissue expresses TH receptor (TR), although at that time fetal thyroid is still unable to produce significant amount of TH. Thus, the fetus is totally dependent on maternal supply of TH at least until midgestation. In fact, it was shown that maternal hypothyroidism during pregnancy results in the reduction of IQ in offspring. Endocrine disruptors (ED) effect on the TH axis through different pathways described below: a) Transthyretin dependent pathway modifies TH transport by lowering circulating TH levels in animals treated with PCB. b) AhR dependent pathway modifies TH degradation through AhR dependant increase of T4 degradation by dioxin. When maternal thyroid is affected by ED, those two mechanisms decrease TH supply to fetus. In neuronal tissue of fetus, c) TR dependent pathway modifies receptor binding and gene activation. d) Other pathways may act through non-genomic effects on neural cell membrane.

Recently, big progress was achieved in the elucidation of the TH dependent pathway. Koibuchi's group examined the effect of T3 and PCB in cultured CV-1 cell transfected with TRE and TR beta 1. When T3 100nM was added, transcriptional activity expressed in terms of luciferase activity was definitely increased. When PCB was added with T3, PCB concentration as low as  $10^{-10}$ M was effective to partially abrogate the effect of T3(1). This system is promising to be employed as a screening system. Kuroda and his associates succeeded in showing the effect of TH in cultured Purkinje cells of cerebellum. They showed the dendritic extension is dependent on the dose of thyroxine ranging from  $5 \times 10^{-12}$  to  $10^{-4}$ M in the serum-free culture medium. Bisphenol A was not effective by itself. However, bisphenol A, simultaneously added with  $5 \times 10^{-9}$  M T4, partially but significantly inhibited the effect of T3(2). His group also succeeded in showing the effect of TH on synaptogenesis in *in-vitro* culture of olfactory nerve cells. He showed that synaptogenesis observed with histochemical method is dependent on the concentration of T4  $10^{-8}$ M. This concentration of T4, not only increase the synapsis formation morphologically, but also increased synapsin-1 formation in the Western blot assay. In his system, synchronization of intracellular Ca oscillation was observed simultaneously with the synaptogenesis. TH dependent increase in synaptogenesis was abrogated by amiodarone which is a TH antagonist. Amitrol showed inhibitory effect on this aspect, while that of BPA, Nonylphenol, and tributyltin was minor, if any(3).

Effects of TH nuclear co-activator or co-repressor were examined by Zoeller's group. They found that in neonatal rat rendered hypothyroid by propylthiouracil showed decreased level of both Co-activator SRC-1 and co-repressor NcoR(4). As these cofactors are shared by different receptor systems, TH regulation of cofactor expression may be important for the ability of other receptor systems during the development.

As one of other mechanisms, non-genomic effects on neural cell membrane were reported by two groups. One is Koibuchi's group describing non-genomic depolarization of cultured neural cells

(5) and the other, Armstrong's group, by employing pituitary cell culture, found that TH stimulates potassium channel activity through a signal transduction cascade that involve PI3 kinase and Rac-GTPase(6).

In parallel with those *in-vitro* studies, an important *in-vivo* study reported that subtle change of maternal TH system causes a permanent alteration in brain cytoarchitecture in the offspring. Hypothyroid rat pups showed less migration of neural cells to the surface(7).

#### **TOP FIVE KEY RESEARCH RECOMMENDATIONS:**

- 1) Screening of endocrine disruptors on the TH dependent neural development by
  - a) reporter gene assay (Koibuchi)
  - b) dendritic extension assay (Kuroda)
  - c) neural synaptogenesis assay (Kuroda)
  - d) cytoarchitecture assay (Lavado-Autric)
- 2) Development of neurogenomic DNA-micro array for revealing the effect of TH or endocrine disruptors
- 3) Examination of cognitive and behavior abnormality of the animal with gestational or lactational exposure to ED.

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**TITLE: Potential Human Health Effects Of Edcs Emphasizing PCBs: Nervous System – Gaps of Knowledge and Research Needs**

**AUTHOR:** Gerhard Winneke, University of Dusseldorf, Medical Institute of Environmental Hygiene, Dusseldorf, Germany, [Gerhard.winneke@uni-duesseldorf.de](mailto:Gerhard.winneke@uni-duesseldorf.de)

The presentation focussed on polychlorinated biphenyls (PCBs) as one relevant group of persistent environmental chemicals with endocrine disrupting properties (EDCs) of the PHAH-family and covered three main topics: (1) the issue of persistence or reversibility of neurobehavioral deficit following early developmental exposure, (2) the possible impact of postnatal exposure through breastfeeding, and (3) interactions with the steroid hormone system in relation to non-reproductive functions.

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE 2002**

The reversibility issue is being discussed since cognitive deficit in relation to pre-perinatal PCB-exposure was reported to be still present in children of the Michigan cohort at 11 years of age (1). More recent reports from three different cohorts are partly at variance with the hypothesis of neurobehavioral PCB-effects to last beyond 4 1/2 or 6 years of age (2, 3, 4). The Dutch study (2) is particularly interesting, because negative effects of prenatal PCBs/PHAHs for subgroups of children from less optimal parental and home environments (younger mothers with lower IQ and poorer education) were reported, although for the full cohort no PCB-related cognitive deficit was observed any more at 6 1/2 years of age, in contrast to observations at 42 months of age (5). This is suggestive of an aggravation of PCB/PHAH-related developmental adversity by social/educational disadvantage.

Since human brain development extends long into the postnatal phase, and since postnatal PCB-exposure of the suckling baby through breastfeeding exceeds prenatal transplacental exposure by orders of magnitude, one would expect a substantial contribution of postnatal PCB-intake through nursing. However, almost all of the available evidence emphasizes the impact of prenatal exposure on postnatal neurobehavioral development (6). The Duesseldorf cohort study (7) was the first to suggest postnatal PCB-exposure via breastfeeding to add to the impact of prenatal exposure on developmental adversity. Since, however, this still is an isolated finding, independent corroboration is needed.

Apart from thyroid effects of PCBs which are discussed as potentially underlying PCB-related cognitive deficit (Shishiba, this symposium), interactions of PCBs with the sex steroid system have also been reported. Sex steroid hormones play a regulating role in brain development. As for non-reproductive functions human data, in contrast to animal data (e.g. 8; 9), are almost missing, so far. Recently, however, questionnaire-based observations were reported (10) showing significantly decreasing mother-reported masculinity (e.g. play-behavior, other male characteristics) in 7 1/2 years old boys with increasing prenatal PCB, and non-significant opposite effects in girls. This is an interesting first report of PCB/PHAH-related gender-specific modulation of brain development in humans, which needs closer attention in future research.

**RESEARCH RECOMMENDATIONS**

- The causal role of thyroid hormones in PCB/EDC-related neurodevelopmental adversity needs further support. Other newly emerging EDCs such as PFOS and PBDEs also need attention here. Experimental (using positive controls) or epidemiological research (e.g. interactions with marginal iodine deficiency or subclinical hypothyroidism) is needed.

- Develop peripheral thyroid-specific markers of response that can be used to diagnose alterations in thyroid hormone functions at critical life stages and can potentially be incorporated into mammalian two-generation reproductive studies.
- Characterize the impact of PHAHs and other emerging EDCs on hormonal systems involved in the determination of sex-role identity and/or gender specific cognitive functions, particularly during brain development. Retrospective cohorts based on archived perinatal tissues are a promising design option here.
- Further clarify the role of social/educational family environments in influencing the appearance or the persistence of neurodevelopmental adversity resulting from exposure to PCBs and other neurotoxic agents (e.g. DDT, metals). The use of enriched vs impoverished environments in animal developmental models should be exploited, as well.

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**TITLE: Human Cancer**

**AUTHOR:** Lizbeth López-Carrillo, Mexico Institute of Public Health, Cuernavaca, Morelos, Mexico, [lizabeth@correo.insp.mx](mailto:lizabeth@correo.insp.mx)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

Increases in hormone related cancer trends can not be clearly explained by improved diagnostic techniques. It has been argued that these trends coincide roughly with the increasing use and release of industrial chemicals, including EDCs, into the environment.

During the last decade results from several epidemiological studies, performed in different countries, were unable to replicate the effect of p,p'-DDE body burden levels and BC risk. In a recent meta-analysis an odds ratio of 0.97 with a confidence interval of 0.87 to 1.09 that means no association between the exposure to this insecticide and breast cancer risk.

There is some evidence that dietary isoflavones protect from endometrial proliferation as shown by a decreased incidence of endometrial cancer in Japanese and U.S. (Hawaiian) women consuming isoflavone-rich diets. Specifically, high consumption of soy products and other legumes was associated with a decreased risk of endometrial cancer . Xenoestrogens as EDCs, might add their effect to natural estrogen, however insufficient human evidence in this regard is available.

In Brazil, moderate to high correlations were observed between ovarian, testicular and prostate cancer mortality respectively. Due to the limitations of ecological studies, this incipient research area deserves more attention.

**TOP FIVE KEY RESEARCH RECOMMENDATIONS:**

1. Evaluation of gene-environment interactions on human cancer.
2. Develop nested case-control studies with core protocols including developed and developing countries,
3. Validation of early biomarkers of damage for epidemiological studies
4. Evaluation of EDCs exposure during the critical periods of development, from conception to adolescence.
5. Determination of individual variations in metabolizing enzymes of EDCs.

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**TITLE: Effects of EDCs in wildlife: Current Status and Research Needs****AUTHORS:** Gerald T. Ankley<sup>(1)</sup> and G. Van der Kraak<sup>(2)</sup><sup>(1)</sup>Mid-Continent Ecology Division, US Environmental Protection Agency, National Health Effects Environmental Research Laboratories, Duluth, Minnesota, USA, [ankley.gerald@epa.gov](mailto:ankley.gerald@epa.gov);<sup>(2)</sup>University of Guelph, Department of Zoology, Guelph, Ontario, Canada, [gvanderk@uoguelph.ca](mailto:gvanderk@uoguelph.ca)**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

There have been marked advances over the past two years in the availability and sophistication of test methods for assessing potential effects of endocrine-disrupting chemicals (EDCs) in wildlife (vertebrates and invertebrates). This has been fueled by the need for tests for regulatory activities; however, it has impacted (decreased) resources/efforts available for documenting occurrence of EDC impacts in the field. Hence, many of the case studies identified some years ago as indicative of EDC impacts in wildlife are still those used as examples of the adverse effects in the field (Ankley and Giesy 1998; WHO/IPCS 2002). Nonetheless, there have been important increases in knowledge relative to these historical situations. For example, feminization of fish by estrogenic chemicals present in municipal effluents continues to be a significant concern and a much wider-spread phenomenon than originally thought a few years ago. In other instances, hypothesized manifestations of EDC-induced effects in the field have been shown likely not to be due to chemicals; for example, it appears that increases in malformations in frogs may be due to biological as opposed to chemical stressors (Blaustein and Johnson 2003). Similarly, hypoxia may be a confounding factor in determining cause and effect relationships. For example, carp exhibited altered hormone biosynthesis under hypoxic conditions, which may contribute to reproductive dysfunction and reduced fertility (Wu et al. 2003).

One group of EDCs that appear to be emerging as an environmental concern are androgen receptor agonists. Studies with both complex mixtures (pulp and paper mill and sewage effluents; feedlot discharges) and single chemicals (e.g., equol) suggest that this class of chemicals could be having significant adverse effects in fish (Parks et al. 2001; Hewitt et al. 2003; Ankley et al. 2003; Orlando et al. 2003; Burnison et al. 2003). Although somewhat contentious, another chemical that has received a significant amount of attention as a possible EDC is atrazine. There are indications that atrazine can alter sexual development in amphibians, albeit the dose necessary to elicit this effect is uncertain (Hayes et al. 2002; Carr et al. 2003).

An important recent emphasis in wildlife studies has been an attempt to better translate the effects of EDCs at the individual level into likely population-level impacts. For example, population models have been developed for fish (e.g., the fathead minnow), and explicitly linked to lab data concerning the developmental and reproductive effects of EDCs to generate predictions of risk in the field (e.g., Brown et al. 2003).

Another area of increasing emphasis is extrapolation of EDC effects across species; for example, in recent studies, Wilson et al. (2004) evaluated conservation of structure and function of the androgen receptor across species. They concluded that, while the effects of androgen receptor agonists/antagonists certainly are expressed differently across species with respect to phenotype, basic similarities in the receptor are conserved, and can defensibly serve as the basis for extrapolation across species.



**KEY RESEARCH RECOMMENDATIONS:**

Focus research on understanding the effects of EDCs across biological levels of organization as a basis for predicting population-level impacts from data collected at lower levels of organization.

Optimize test methodologies to relate effects at the level of the individual to alterations in specific endocrine pathways; emerging genomics technologies are one promising approach to doing this.

Continue to broaden the focus on EDCs from more than just estrogenic mediated effects. Other relevant modes/mechanisms of action deserving attention include chemicals that may affect other signaling pathways (e.g., androgen, thyroid, retinoid, prostaglandin, corticosteroid) that impact growth, reproduction and development.

Focus on understanding extrapolation of EDC effects across species as a basis for more effective prediction of risk for all species; here again, new molecular biology approaches could be very fruitful.

Develop approaches using in silico models and in vitro systems that can serve as effective screens for EDCs, e.g., to help prioritize chemicals for more costly whole-animal testing.

Develop improved exposure assessment models that incorporate both the measurement of EDCs in the environment and at target tissues and consideration of confounding factors in order to establish cause and effect relationships.

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**TITLE: Recent Developments on Endocrine Disruptors Testing and Assessment at the OECD Level**

**AUTHOR:** Anne Gourmelon, Environment, Health and Safety Division, Organisation for Economic Co-operation and Development, Paris, France, [anne.gourmelon@oecd.org](mailto:anne.gourmelon@oecd.org)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER 2002**

The Organisation for Economic Co-operation and Development started a Special Activity on Endocrine Disruptors Testing and Assessment (EDTA) in 1998, at the request of member countries to ensure that test methods and strategies developed for endocrine disrupting chemicals would not differ substantially among countries. The OECD has on its work programme the development and validation of several test methods for the detection and characterization of these substances. The EDTA Task Force supervises the work on testing and assessment; it is composed of representatives from OECD countries, industry and NGOs. The area of human health effects, represented by rodent tests, is more advanced: the validation of test methods is finalized for the Uterotrophic assay (Owens and KoMer 2003) and well underway for the Hershberger bioassay (Yamasaki et al. 2003) and the enhanced Test Guideline 407.

In the area of testing with environmental species, there was no existing Test Guideline that could easily be enhanced for the detection of endocrine disruptors. It took longer to discuss and agree on the basis on which to build test methods that would fit the purpose. The fish screening assay for the detection of endocrine active chemicals with various modes of action is in the process of being validated. The intent is to be able to use it *i*) for the detection of estrogenic, (anti-)androgenic substances and aromatase inhibitors, and *ii*) to set up priorities for further testing. The development of the fish screening assay is based on work conducted in the United States (Ankley et al. 2003), Europe and Japan. It makes use of adult fish in spawning condition, exposed for 21 days to test substances. Vitellogenin, spawning status, secondary sex characters and gonadal histology are the core endpoints measured. Considerable efforts are being made to establish a relevant and reliable test, with three of the most commonly used species in regulatory context around the world.

Other fish tests of interest span over longer periods of time and focus on sensitive life-stage and transgenerational effects. This is the case of the fish early life-stage (Andersen et al. 2003; Orn et al. 2003) and fish full life-cycle tests (Seki et al. 2003). The latter aims at defining whether endocrine active substances identified in screening will have effects on growth and reproduction and will affect fish populations. Diverse approaches exist, once countries have a better understanding of advantages and limitations of each approach, a standardization of the preferred one can be foreseen, as the first step towards validation.

For the detection of thyroid effects, the amphibian metamorphosis model holds promises (Kloas 2002). It needs to be checked through testing with similar substances as in mammalian tests whether the amphibian model is reflective of thyroid effects in vertebrates in general and whether the assay covers diverse thyroid disruption pathways measurable at the organism level.

It has been more challenging to define the scope and objectives of testing and assessment of endocrine disruptors in invertebrates. This can be explained by *i*) the fact that invertebrates represent a huge biodiversity, also implying diversity of endocrine systems, and *ii*) the absence of profound knowledge of these invertebrate endocrine systems, with the consequence that

endocrine specific endpoints are difficult to identify. A number of development and reproduction tests with invertebrates already exist (Breitholtz and Wollenberger 2003; Hutchinson et al. 1999) for the general ecotoxicology and the use of such tests within a testing strategy for endocrine disruptors is being discussed by member countries.

#### TOP FIVE KEY RESEARCH RECOMMENDATIONS:

1. Improve basic knowledge of the biology of species used in screening and testing programs, so that there are benchmarks for evaluating the homeostasis or normal variability of endpoints measured compared to a significant response/effect after chemical exposure and for building stronger foundation of extrapolation across species.
2. Identify endpoints in the amphibian metamorphosis assay indicative of thyroid-related disruption pathways that might be used to extrapolate findings to vertebrates. This will necessitate the parallel assessment of chemicals in the various screening tests for thyroid effects.
3. Evaluate the need for multigenerational versus full- or partial-life cycle test designs for chemical hazard assessment of the EDCs in fish.
4. Standardize emerging technologies (e.g., biomarker protein measurement methods) for screening purposes.
5. Develop a better understanding of predictivity of newly developed methods (not only in ecotoxicology testing, but in QSARs as well).

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**TITLE: Critical Research Needs for Screening and Testing Methodologies**

**AUTHOR:** George P. Daston, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati, Ohio, USA, [daston\\_gp@pg.com](mailto:daston_gp@pg.com)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER 2002:**

The development and validation of screening and testing methods for endocrine disrupters is well underway. Many of the new findings involve reports on the state of validation of screens. Of particular note is the large effort by OECD to validate a standardized protocol for the rodent uterotrophic assay to detect estrogens. This program is largely finished, and there have been several publications that describe the results of the program (Owens et al., 2003; Kanno et al., 2003a, b; Owens and Koeter, 2003). Similar progress on the Hershberger assay for androgens is expected in the next year. Another noteworthy activity is the publication of the U.S. ICCVAM's report on the state of validation of in vitro methods for detecting estrogens and androgens (ICCVAM, 2003). This report was somewhat of a surprise in that it concluded that although receptor binding assays for estrogens and androgens have been in use for decades, there is no standardized method. One will need to be developed and validated before this aspect of screening can be initiated. The state-of-the-art for transcriptional activation assays is even less advanced and will require further methods development. Finally, research in genomics has started to address a deficiency in the proposed endocrine disrupter screening battery, i.e., the lack of representation of early development. Transcript profiles have been published for estrogens in the rodent fetus and compared with those of the mature uterus (Naciff et al., 2002, 2003).

**TOP RESEARCH RECOMMENDATIONS:**

1. Standardize and validate methods for receptor binding assays for estrogens and androgens: Detailed recommendations are provided in the ICCVAM report cited above. Most importantly, we should take advantage of the availability of human recombinant receptors. Not only are these the most relevant for human health, but recombinant proteins provide a standard source of pure receptor, and individual isoforms can be studied. Because of the extensive literature on receptor binding assays, standardization and validation will proceed quickly once it is started.
2. Methods development for transcriptional activation assays: Again, the ICCVAM report provides detailed recommendations. In general, the replicability, dynamic response, and specificity (especially for androgens) of these assays needs to be improved in order to be useful for screening.
3. Continue to develop gene-based approaches for screening assays in developing systems: One of the stated principles for endocrine disrupter screens is that they be sensitive, but the most sensitive lifestage (at least putatively) is not represented in current screening schemes. Recent work demonstrates the feasibility of identifying specific transcript profiles in fetal tissues for endocrine disrupters. This work should continue.
4. Develop a test method for resolution of positives in the screening battery that is less extensive than the multi-generation assay: The screening tier has been designed to be highly sensitive and the results are likely to be skewed towards producing many false positives. At present the only means of resolving a positive in the screening tier is a

- multigeneration test. These assays are so lengthy and resource-intensive that there is likely to be a large backlog of chemicals to be tested. Therefore, for purely pragmatic reasons it would make sense to design a more streamlined assay that could resolve positive results in the screening tier.
- Retrospectively analyze the reproductive and developmental toxicity database to determine whether they are suitable for resolving whether a substance is an endocrine disrupter: Although older reproductive and developmental toxicity studies have been criticized because they did not include endpoints that were specific for endocrine disrupters, they do include a large number of measurements known to be responsive to EDCs. A retrospective analysis of these studies to determine the extent to which they detected a signal when an EDC was tested might forestall the need to re-test chemicals that have otherwise been extensively evaluated in traditional toxicity batteries.

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**TITLE: Critical Data Gaps / Research Needs –Methodologies for Assessing EDCs  
Genomic Approaches**

**AUTHOR:** Jun Kanno, MD, PhD Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan, [kanno@nihs.go.jp](mailto:kanno@nihs.go.jp)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

- A. Concept of phenotype-independent toxicogenomics and its necessity
1. Endocrine Disrupting Chemicals Issue
    1. Homeostatic regulation will cancel out exogenous hormonal stimuli in very high efficiently.
    2. Hypothalamus-Pituitary-Gonad Axis must be working at molecular level.
    3. Have we ever looked at such biological responses in terms of toxicity (adverse effect)?
  2. To reduce Attrition in Drug Development
    1. Example: Thalidomide story: An old story?
    2. Malformation was induced in humans, not in rats and mice, but was reproduced in rabbit
    3. Recent literatures shows that Thalidomide works in mice, inducing gene expression even in liver!
    - 4.
  3. Modernization of Risk assessment in general
    1. Do you use the concept of “uncertainty factor” forever?
    2. How long do you keep using the concept of “LD50”?
    3. Mechanism-based modernization is needed
  4. Where to look at biological responses in terms of molecular toxicology
    1. Final product cascade
      1.  $x10 \sim x20 \sim$
      2. direct links to phenotype
    2. Signal cascade
      1.  $x1.5 \sim x2$
      2. no direct links to phenotype
  5. “No direct links between mRNA expression profile and toxicity (phenotype)”. All genes are equally important! Larger comprehensive database is essential
    - Ratio data are not adequate → absolute readouts are optimal
    - We don’t know which genes link to toxicity → need to look all of them
    - Qualitative change is not enough → good dose-response is needed
  6. Absolutization (percellome) (Absolute monitoring of mRNA levels)
    1. Concept: Obtain mRNA quantity data in a “per one cell” basis.
    2. Merits:
      - Accurate comparison among samples showing radical changes in mRNA synthesis where house keeping genes are not constant and global adjustment becomes unreliable (time course studies, dose-response studies, etc.)
      - Accurate comparison between separate studies
      - Accurate comparison between different microarray (different version, different make)



## B. Application of Toxicogenomics to EDC issue, Screening &amp; Testing and Definitive Testing

- Scheme for ED screening and testing (MHLW)
  - Prioritized List Of Chemicals for Defenitive Testing
    - ◇ *In silico* Screening (ERa, ERb,AR, TR, AhR, etc)
    - ◇ *In vitro* Screening(ERa, ERb,AR, TR\*, AhR\*\*, etc)
    - ◇ *In vivo* Screening(ER, AR, TR, AhR, etc)
    - ◇ Possible addition to the scheme: Pathway Screening: **by Toxicogenomics method**
- Proposal of a conceptual protocol of “One life-span assay of rodents” for endocrine disruptor testing
  - Mechanistic studies supported **by Toxicogenomics method**

## C. Conclusion

- Toxicogenomics,
  - of phenotype-independent approach can be used as a measure to depart from Regression model-based (fingerprint-based) toxicology to Signal cascade based (mechanism of action-based) Toxicologenomics.
  - can be used as EDC “Screening backup” and “Testing backup”
  - cab be used as a powerful measure to improve EDC “Definitive testing”
  - in the future, can modify the existing methods / protocols

**TOP FIVE KEY RESEARCH RECOMMENDATIONS:**

- 1 Toxicogenomics of phenotype-independent approach can be used as a measure to depart from Regression model-based (fingerprint-based) toxicology to Signal cascade based (mechanism of action-based) Toxicologenomics.
- 2 Toxicogenomics can be used as EDC “Screening backup” and “Testing backup”
- 3 Toxicogenomics cab be used as a powerful measure to improve EDC “Definitive testing”
- 4 Toxicogenomics , in the future, can modify the existing methods / protocols

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**TITLE: Recent Progress Environmental Exposure to Endocrine Disrupting Substances**

**AUTHOR:** Mark R. Servos, Department of Biology, University of Waterloo, Ontario, Canada, mservos@uwaterloo.ca

**SIGNIFICANT NEW RESEARCH FINDINGS:**

The high diversity of potential endocrine disrupting chemicals (EDC) in the environment makes the exposure assessment of this group a continuing challenge. However, there continues to be a significant international effort to document and understand the exposure of these substances to both humans and ecosystems. Many of the persistent organic contaminants, such as selected organochlorine pesticides, polychlorinated Biphenyls (PCB) and polychlorinated dibenzo-p-dioxins (PCDD), that traditionally have been associated with endocrine disruption, continue to be widespread in the environment as was outlined in the WHO Global Assessment Report. Despite global efforts to eliminate many of these compounds, they continue to be detected in the environment and human diets. Research also shows that these chemicals may be associated with a variety of potential endocrine related biological consequences in ecosystems and humans, although in most cases it remain difficult to make a specific cause and effect linkage (e.g. Harkins et al. 2002; Perera et al. 2003; Shaw and McCully 2003). These persistence substances remain widely distributed in even remote environments such as the arctic (AMAP 2003). Of particular concern is the continued elevation of environmental contaminants such as polybrominated diphenyl ethers (flame retardants) that are detected in mother's milk (Mazdai et al. 2003; Schecter et al. 2003) and have increased in recent years in environments such as the Great Lakes, as well as the arctic (Ikonomou et al. 2002). Large monitoring programs have been initiated to evaluate the exposure of EDCs, including phytoestrogens, to sensitive populations of humans, especially children (e.g. Landrigan et al. 2003) and sensitive ecosystems such as the arctic.

Recent studies have indicated that industrial effluents contain a wide variety of potential EDCs. Even with significant process changes and improvement in pulp and paper mills reproductive effects are still seen at many site associated with these effluents (e.g. Parrott et al. 2003). The removal of elemental chlorine resulted in the removal of chlorinated chemicals such as PCDDs but many subtle effects on the reproductive performance of biota, such as fish remain. Efforts continue in an attempt to identify the substances responsible for these effects (e.g. Hewitt et al. 2003). However, these complex mixtures likely contain a variety of chemicals that impact reproduction and development through a diversity of mechanism including estrogenic, androgenic and thyroid systems (e.g. Ellis et al 2003; Alsop et al. 2003) making the isolation of the causative agents very difficult. Considerable progress is being made but identification and remediation of the causative agents in these effluents remains a major challenge.

Municipal effluents have been identified as having the potential to alter endocrine function in fish exposed to the outfalls. Considerable effort has focused on identifying the responsible chemicals in these effluents. Numerous potential EDCs, including natural and synthetic estrogens, bisphenol A and degradation products of alkylphenol polyethoxylates have been isolated in these effluents. The relative importance of these chemicals in different effluents appears to be dependent on the degree and type of treatment. Secondary treatment is effective at removing some but not all of these substances. Treatment changes the relative distribution of these substance found in both effluents and sludges making it very difficult to assess or predict the fate and risk (Ferner et al. 2002). Most of the studies have been associated with the estrogenic responses but studies are now indicating that several of these chemicals, including degradation products of alkylphenols,

and additional compounds (e.g., testosterone) may also exert androgenic effects (Chattopadhyay et al. 2003). A very complex mixture of chemicals are released into the environment from municipal treatment sites that have implications for environmental health. Recent studies have examined the processes that may potentially be important in removal of these substances in municipal effluents as well as drink water treatment plants (Ternes et al. 2002) but there remain considerable work to be done to implement appropriate risk management steps.

A rapidly emerging area of concern is the detection pharmaceuticals and personal care products (PPCP) in the environment (Daughton 2003). A number of these compounds may have the potential to disrupt endocrine function in both vertebrates and invertebrates through a variety of mechanisms. A wide diversity of these chemicals have been detected in effluents, surface waters and even drinking water sources (Daughton and Ternes 1999; Koplín et al. 2002; Metcalfe et al. 2003). A wide variety of PPCPs may also enter the environment through the agricultural use of biosolids or the spreading of animal manures which may contain both natural hormones or pharmaceuticals used to treat animals or promote growth. Burnison et al. (2003) isolated natural hormones as well as the phyoestrogen degradation product equol, in the manure and runoff from treated fields. Studies have also recently identified the presence and potential effects of growth promoters near intensive animal production facilities (Orlando et al. 2003). Trenbolone detected in adjacent water courses has been shown to have the potential to cause androgenic effects in fish (Ankley et al. 2003).

Assessing the exposure of endocrine disrupting substances remains very difficult due to the wide variety of chemicals, diversity of sources, environmental degradation and fate. In many cases the chemicals responsible for effects have not yet been identified or methods are not available to quantify them. Bioassay directed toxicity identification evaluation approaches may be very helpful in identifying specific chemicals or focusing efforts on specific groups of chemicals, especially if the endpoints can be directly linked to the effects of concern observed in the environment. Alternate approaches using biomarkers or indicators of exposure may also provide useful tools for supporting or directing exposure assessments. A considerable amount of work remains to be done to adequately define the exposure of human population and ecosystems to endocrine disrupting chemicals.

#### **RESEARCH RECOMMENDATIONS:**

1. Temporal and spatial aspects of the exposure, i.e. selected sensitive life-stages and populations.
2. Complex mixtures: Identification of causative agents, additive effects, cumulative exposure.
3. Developing chemical and biological analytical methods for assessing exposure, especially emerging issues.
4. Devising methods and techniques for treating wastes (municipal, industrial and agricultural) and best management practices (intensive agriculture) to reduce the release of EDCs.
5. Developing the knowledge to link exposure, effects and risk.

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**Title: Exposure of wildlife to endocrine disrupting chemicals**

**Author:** Masatoshi Morita, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan, [mmorita@nies.go.jp](mailto:mmorita@nies.go.jp)

**SIGNIFICANT NEW RESEARCH FINDINGS SINCE SEPTEMBER, 2002**

The concentrations of selected endocrine disrupting chemicals (EDCs) in various environmental media (air, water, soil, food) have been measured over recent years. The development of analytical methods, especially GC/MS techniques (GC/HRMS, GC/CINI-MS) and LC/MS(/MS) has made it possible to determine hitherto undetermined EDCs including non-volatile metabolites. Data relevant to internal exposure (blood, milk, tissue) are still limited both for humans and wildlife. Available data have resulted from a focus on persistent bioaccumulative chemicals, mainly organochlorine compounds. However, several other chemicals, such as brominated compounds (PBDE, PBDDs/DFs) and fluorinated compounds (PFOS), have recently attracted attention. Analysis of PCDD/DFs and PFOS is now well advanced and it is possible for analysis to be done on only 5 ml of blood serum for the normal population. EDCs that are easily degraded in the environment or organism may not be detected at the time that adverse effects, such as reproductive deficit, became apparent. The question remains as to which chemicals are potentially important as EDC(s) and should be investigated in the environment.

The application of high-throughput bioassay methods (receptor binding assays, cell proliferation assays, etc) has become more frequently used to assess hormonal potency in the environment. The measurement of estrogenic potency, for example, is now well established for water in relation to the feminization of fish. Sensitivity depends in part on the receptor used and, therefore, it is necessary to know the receptor characteristics of the target animal species. In some cases it is considered that the hormonal potency of water can be explained by the sum of the estrogenic potency of the chemicals analyzed by instrumental analysis. However, in most cases care should be given to the evaluation of such results because of the presence of synergist(s) and antagonist(s) in real samples. Separation techniques and the use of a combination of assay techniques may help to solve the problem.

Field studies have been implemented in individual cases where endocrine disruption is suspected, and it has been shown that exposure to certain EDCs has contributed to adverse effects resulting in population decline in several wildlife species. Example of Japanese studies include the following:

Birds: It has been clearly demonstrated that birds-of-prey and fish-eating birds accumulate persistent organochlorine compounds including PCBs, PCDD/DFs and DDT at significantly high concentrations. Two species, the oriental stork (*Ciconia boyciana*), and the crested ibis (*Nipponia nippon*), are already extinct and one species, the great cormorant (*Phalacrocorax carbo*) had its population reduced in the 1970s. The great cormorant is an example of a bird species that has recovered from the edge of extinction. The responsible chemicals are probably PCDD/DFs and, in part, PCBs because residue levels of these chemicals are still high and the time course of its population size is inversely correlated with the environmental contamination levels of PCDD/PCDFs (Iseki et al., 2001). Thyroid hyperplasia is still widely seen in birds of the Tokyo Bay area suggesting that the subtle effects of PCDD/DFs continue to be felt (Ministry of Environment, Japan).

Fish: Exposure to estrogenic compounds is known to cause feminization in fish with different effect levels, from the induction of yolk proteins such as vitellogenin in male fish to the creation of intersex or sex change, depending on the exposure levels. Japanese national surveillance was carried out on carp in fresh water and on several marine fish species in coastal areas. Slight vitellogenin induction is widely observable in populated inland and coastal areas, and stronger effects can be seen close to sewage treatment plants. Causative chemicals are considered to be natural estrogens such as estradiol and estrone of human origin and, in part, phenolic compounds such as nonylphenol and bisphenol-A that are manufactured in large amounts and used for domestic purposes.

Invertebrates: Exposure to tributyltin (TBT) or triphenyltin (TPT) has caused the masculinization of females and resulted in population decline of some gastropod mollusk species. More than 140 species of gastropods are reported to show such effects (Matthiessen et al., 1999). A cause-effect relationship is clear in this case and a structure-activity relationship is well established; tri-substituted organic tin compounds with appropriate organic groups are the most effective (Matthiessen et al., 1999). After the ban of these chemicals for use in antifouling paints, the level of imposex is declining and the populations appear to be recovering for some species, but the problem remains because of the persistent nature of the chemicals in sediments and, in part, by the unregulated emission of the compounds from overseas vessels. Several hypotheses have been proposed to explain the imposex effect, i.e., the development of male sex organs in females. These include aromatase inhibition, alteration of neuro-peptide hormones and others. We are now presenting a new hypothesis that the retinoid X receptor is involved (Nishikawa et al., 2004). TBT/TPT binds strongly to the RXR receptor and 9-cis-retinoic acid (the natural RXR receptor ligand) induced the development and growth of male sex organs in female gastropods in an analogous manner to the imposex caused by TBT or TPT (Nishikawa et al., 2004).

There are other cases of adverse effects observed in certain species that are likely to be endocrine mediated, but in most instances the causal link between chemicals exposure and endocrine disruption remains unclear. Further effort is necessary to understand the phenomena. Evidence for effects resulting from exposure to EDCs requires information on exposure levels, the effect/exposure relationship, other modulating factors, bio-statistics, the time courses of exposure and effects and others. The preparation of a database of candidate or potential EDCs will help the development of understanding.

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**TITLE: Current and Future Research in the Endocrine Disrupter Field in the European Union**

**AUTHOR:** Dr Tuomo Karjalainen, Scientific Officer, European Commission, Research Directorate-General, Directorate E: Biotechnology, Agriculture and Food, Food Quality, Brussels, Belgium, [tuomo.karjalainen@cec.eu.int](mailto:tuomo.karjalainen@cec.eu.int)

**SIGNIFICANT NEW RESEARCH SINCE SEPTEMBER, 2002**

The Directorate-General for Research of the European Commission implements the recommendations published the Community Strategy on Endocrine Disrupters adopted in 1999 (1). It called for further research, international co-ordination, and communication to the public, when addressing the complexities of endocrine disruption.

DG Research regularly publishes calls for proposals for research projects, which are composed of teams from several European (or infrequently extra-European) countries. In the 5<sup>th</sup> Framework Programme of Research (1998-2002), the Quality of Life Thematic Programme has spent over 43 million euros on ED projects and has sponsored close to 20 large projects, which are still ongoing and results are to be expected in the next couple of years (2). The Energy, Environment and Sustainable Development Programme has financed projects focused on wild-life effects (budget of around 16 million euros) (2). In 2001, the two programmes launched a joint call focused on endocrine disrupters, culminating in the formation of the 63-laboratory, 4-project CREDO (the Cluster of Research into Endocrine Disruption in Europe) cluster, which was formally launched in March 2003 (3). 6 other research projects, launched in 2002 and 2003 (2, 3) are associated to the cluster. The projects financed by the 5<sup>th</sup> Framework Programme mainly address (i) development of analytical methods, biosensors and biomarkers; (ii) monitoring of endocrine disrupters in the environment; (iii) fish and invertebrate endpoints; and (iv) exposure of humans to endocrine disrupters and possible health effects (including low-dose and combined effects).

**FUTURE RESEARCH**

Endocrine disruption has not been forgotten in the Sixth Research Framework Programme (2002-2006) (4), the topic being specifically addressed by Priority 5 (Food quality and safety) (5); and, to a lesser extent, by Priority 6 (Sustainable development, global change and ecosystems) (6). In Priority 5, endocrine disruption is covered in particular by the sub-area “[Environmental health risks](#)” where one research focus is “Impact of Endocrine Disrupters”. ED is also covered under the sub-area “[Methods of analysis, detection and control](#)”, which includes methods and standards for analysing and detecting chemical contaminants.

The first call for proposals launched in 2002 resulted in the funding of a 5-year Network of Excellence called CASCADE (Chemicals as contaminants in the food chain: an NoE for research, risk assessment, education, and information), with a budget of 14.4 million € and 18 European organisations participating (7). The aim of this project is to provide European consumers with reliable information on health risks associated with exposure to chemical residues in the food. The research component of this project is focused on how food components and contaminants modulate nuclear receptors leading to disease development (cancer, obesity, osteoporosis, reproductive problems...). This NoE will have numerous activities open to outside participants (open fora, PhD programmes, fellowships, summer schools on risk assessment and nuclear receptors etc). The project was launched in February 2004. A 3-year Specific targeted Research

project called DEVNERTO (Toxic threats to the developing nervous system: *in vivo* and *in vitro* studies on the effects of mixture of neurotoxic substances potentially contaminating food) has also started recently.

The second call for proposals (deadline 5 February 2004) is looking for proposals on “Environmental and endogenous factors influencing puberty onset” and “Food and fecundity”. Specific Support Actions (e.g., workshops, database building) are solicited concerning “Initiatives to improve international coordination of research in the field of endocrine disruption”. Evaluation will take place in the spring of 2004.

Priority 6 in its 2<sup>nd</sup> call for proposals is looking for research projects on “Development of risk assessment methodologies” for, e.g., pesticides, biocides, and pharmaceuticals. Evaluation of projects is ongoing.

New calls will be launched in 2004 and topics include those of interest to the international ED community (workprogramme is under preparation). Non-EU countries can participate in EU research projects as full partners and can even receive funding. Such collaborative projects would be beneficial to all parties concerned to avoid duplication of research efforts. In addition, experts are looked for to evaluate research projects (for registration see [8]).

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**TITLE: USEPA's Endocrine Disruptors Program: Ongoing National and International Collaborative Research Activities**

**AUTHOR:** Elaine Z. Francis, US Environmental Protection Agency, Washington, D.C., USA, [francis.elaine@epa.gov](mailto:francis.elaine@epa.gov)

**NATIONAL AND INTERNATIONAL RESEARCH COLLABORATIONS:**

The magnitude of the scientific uncertainties about the causes, effects, exposures, and solutions to address the concerns regarding endocrine disruptors require national and international coordination and communication. To facilitate efforts nationally, the US President's National Science and Technology Council established an interagency working group on endocrine disruptors. This working group was chaired by the Environmental Protection Agency (EPA) and included representation from 13 other federal agencies. The working group met from 1995 through 2000 and was reconvened in 2003. The first working group developed a national framework for research, an inventory of federal research programs, identified high priority research gaps, and served as a vehicle through which two multi-agency solicitations for research proposals were developed and issued to help fill in some of these gaps. The current working group recently met for the first time to begin to identify its agenda for the coming year.

At an international level, EPA has been working bilaterally and multilaterally to ensure that there is coordination and communication regarding research and testing programs for endocrine disruptors. EPA has ensured that endocrine disruptors has been on the agenda of the annual G-8 Environmental Ministers' Meeting, as part of the mutual interest in children's health, annually since 1997. At the 1997 meeting, the Ministers recommended that a global endocrine disruptors research inventory be developed and that a global state of the science report be prepared. EPA chaired both of these efforts. EPA has been working with the European Union and with Japan's Ministry of the Environment to promote scientific collaborations by co-organizing workshops. Furthermore, EPA has been participating on work groups under the auspices of the Organization for Economic Cooperation and Development to develop, validate, and harmonize screening and testing guidelines. Other internationally-related EPA activities include: 1) having discussions with the European Union (EU) regarding the possibility of issuing a simultaneous coordinated solicitation for research proposals, 2) supporting, through an extramural grants program, US institutions who are studying endocrine disruptors in populations in other countries, 3) participating in twice yearly US-EU Science and Technology Meetings, where endocrine disruptors has been a topic on the agenda, 4) sharing information on our research and testing programs with scientific delegations from other nations that visit EPA, and 5) promoting individual collaborations between scientists at EPA and other countries.

**TOP RECOMMENDATIONS FOR FURTHER INTERNATIONAL COLLABORATIONS:**

- Identify, support, and organize joint international workshops on focused topics.
- Consider the development of the next generation of a global inventory of projects.
- Promote the exchange of scientists.
- Work through countries' or agencies' international affairs offices to build on existing bilateral and multi-lateral efforts (e.g., through ongoing science and technology agreements).

- Consider establishing an international research committee on endocrine disruptors (through governmental organizations), using existing models.