Endocrine disruptors and environmental impact in Japan

Introduction

Various chemical substances are used in business, industries, agriculture and our daily life. It is an important subject to prevent hazards to our health and living environment resulted from environmental contamination by toxic substances. In Japan, we have experienced the contamination caused by harmful chemical substances, such as methylmercury cadmium, PCB in the wastewater from chemical plants. The atmospheric pollution by dioxin, which are formed unintentionally as byproducts from waste incineration and diesel exhaust, also affects the people in the area. Recent investigations suggests that a number of man-made chemicals ranging from plastics products to pesticides may interact with the endocrine system of humans and wildlife populations. Many of them are known by their high toxic levels in animal studies, but not scientifically determined the low level toxic effects in the environment on humans and ecological systems.

Research projects

Many relating projects were recently started in government institutes, universities and industries. So serious is the problem that it could well threaten the very survival of mankind itself. Japanese Environmental Agency completed and presented the *Strategic Program on Environmental Endocrine Disruptors '98* (SPEED '98) to the public in May 1998. Based on these programs, studies were started throughout Japan to determine the extent of environmental pollution and its effects on wild life. Other Japanese governments, such as Ministry of Health and Welfare (MHW), Ministry of Agriculture, Forestry and Fisheries (MAFF), Ministry of Construction (MOC), and local governments also started studies on this subject. Relating reports are provided via World Wide Web (WWW) (Table 1).

MHW started work on this issue in 1996 and engaged in research on the *High Throughput Pre Screening Methods* (HTPS), on mechanisms relating to human health and test methods, and on exposure of foods and containers etc. to fetuses and adults. Examples of references are:

Exposure to endocrine disrupting chemicals: Ema et al. (1999a, 1999b, 2000), Hirose et al. (1999), Ikeda et al. (1999), Nagao et al. (1999a, 1999b, 2000a, 2000b), Ohta et al. (2000), Shirai et al. (1999), Tamura et al. (1999).

Test methods: Akimoto et al. (2000), Minegishi et al. (1997, 1998), Nagasaki et al. (1999a, 1999b), Nakagomi et al. (1999a, 1999b), Ohkura et al. (1999a, 1999b), Ohno et al. (1999), Suzuki et al. (1999a, 1999b).

Rough outlines of these works were shown in Table 2.

The Advisory Committee on Health Influence of Endocrine Disrupting Chemicals was established in April of 1998, in order to examine problems of endocrine disrupting chemicals. An interim report was published in November of the same year and provided via WWW (Table 1).

The Japan Society of Endocrine Disruptor Research, comprised of scientists and engineers from various different fields, was established in June 1998 to shed light on the scientific aspects of the problem. The background of the member scientists is spreading in a wide variety including medical, biological, fisheries, environmental sciences and engineering as well. The International Symposiums on Environmental Endocrine Disruptors were held at Kyoto in December 1998 and in Kobe in December 1999. The main topics in the symposiums and related meetings were: (a) *Screening Methods and Cellular Mechanisms*; (b) *Testing and Hormone Responses*; (c) *Mechanism of Action*; (d) *Fish Testing*; (e) *The Effect of Endocrine Disruptors (ED) on Humans*; (f) *The Effect of EDs on Wildlife*; (g) *Dose-response*; (h) *Potential Effects on Human Health*; (i) *Basic Biology*; (j) *Pesticide*; (k) *Activities in Japan*.

Rough outlines of these works were shown in Table 2.

Database

One of the best ways to provide information widely is the use of Internet, especially WWW. Although English is necessary for international information exchange, Japanese is easier for domestic people. In the Home Page of National Institute of Health Sciences (NIHS), the information on Drugs, Food, Chemicals and Environment are represented. Recently, *Endocrine Disruptor Information Guide for Researchers* was included in the Topics of NIHS Home Page (Table 1). Although it is not yet completed, the *Search Engine: (Web search for Endocrine Disruptors)* and *Lists of Paradigmatic Chemicals* were available.

In NIHS, we have been developing related databases, such as *Endocrine Disruptor Structure Database* (EDSD) (Nakano et al., 1998), *Binding Affinity Database for Endocrine Disruptor* (BADB) (Kaminuma et al., 2000) and *Receptor Database* (RDB) (Nakata et al., 1999). In EDSD, chemical names, CAS registry numbers, synonyms, physicochemical properties and two-dimensional and three-dimensional structural data of 149 substances were included. With appropriate viewing software, users can generate three-dimensional images of chemicals. In BADB, experimental data for interaction of exogenous chemicals and bio-molecules were stored. At present, 742 Competition Binding Experimental results and 376

Table 1

Selected international institutions providing information on endocrine disruptors.

Contents	URL
Ministry of the Environment	
Strategic Program on Environmental Endocrine Disruptors '98	http://www.env.go.jp/en/pol/speed98/sp98.pdf
Trend of countermeasure for pollutant (in Japanese)	http://www.env.go.jp/chemi/sesaku/ehs_idx.html
Interim reports by Research Group on EDs	http://www.env.go.jp/chemi/end/index.html
National Institute for Environmental Studies	
Pesticide Database (in Japanese)	http://info.nies.go.jp:8093/kis-plus/n_start.ASP
National Institute for Minamata Disease	http://www.nimd.go.jp/english/index.htm
Research reports (in Japanese)	http://www.nimd.go.jp/nimd_kenkyu.html
Ministry of Agriculture, Forestry and Fisheries	
The outline of research projects in Agriculture, Forestry and Fisheries (in Japanese)	http://www.s.affrc.go.jp/docs/hyouka/kadai/h10/zen4.htm
Ministry of Health, Labour and Welfare	
Measures for the problem of dioxine (in Japanese)	http://www.mhlw.go.jp/topics/dioxin/index.html
Advisory Committee report on Health Influence of Endocrine Disrupting Chemicals (in Japanese)	http://www1.mhlw.go.jp/shingi/s9811/s1119-2_13.html
National Institute of Health Sciences	http://www.nihs.go.jp
Endocrine disruptors (in Japanese)	http://www.nihs.go.jp/hse/endocrine/index.html
Endocrine Disruptor Information Guide for Researchers	http://www.nihs.go.jp/hse/endocrine-e/index.html
Global Information Network on Chemicals	http://www.nihs.go.jp/GINC/index.html
Tokyo Metropolitan Government	
Dioxine information (in Japanese)	http://www.kankyo.metro.tokyo.jp/dioxin.htm
Endocrine disruptors information (in Japanese)	http://www.kankyo.metro.tokyo.jp/horumon/edc_top.htm
Tokyo Metropolitan Research Laboratory of Public Health	
Biological Effects Database of EDs (in Japanese)	http://www.tokyo-eiken.go.jp/edcs/edcs_index.html
Potential EDs Data (in Japanese)	http://www.tokyo-eiken.go.jp/topics/endocrin.html
About EDs (in Japanese)	http://www.tokyo-eiken.go.jp/edcs/naibunpi.html
Tokyo Metropolitan Research Institute for Environmental Protection	
About EDs (in Japanese)	http://www.kankyoken.metro.tokyo.jp
Kanagawa Environmental Research Center	
Information on EDs (in Japanese)	http://www.fsinet.or.jp/~k-center/hormone/hormone.htm http://www.fsinet.or.jp/~k-center/hormone/contents.htm
Information on chemicals and KIS-NET (in Japanese)	http://www.fsinet.or.jp/~k-center/chem/contents.htm

Table 2

Selected endocrine disruptors studies, 1998-2000.

Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Visser	1998/ISEED '98, pp. 18-21*	OECD	The work of OECD on testing and assessment of ED	Review	The object of the OECD ED activity are to: (i) provide information and co-ordinate activities, (ii) develop new and existing test Guidelines to detect ED, (iii) harmonize hazard and risk characterization and assessment approaches for ED among member countries
Screening Methods and Cellular Mechanisms					
Maciorowski	1998/ISEED '98, pp. 22-23	United States	ED screening and testing in the United States: a consensus based approach to regulatory implementation	Review	EDSTAC was charactered by EPA to provide advice concerning the development of a strategy for screening and testing EDC. The committee formulated a series of recommendation; a conceptual framework and related guiding principles, strategy for sorting and prioritizing chemicals, etc.
Lubahn	1998/ISEED '98, pp. 24-25	United States	Responses to environmental estrogen in ER α KO mice	Ovariectomized wild type and ERα-knockout mice were treatment with MXC or estradiol-17_β. Competitive pretreatment	A xenoestrogen, MXC, can increase LF mRNA concentrations by a mechanism that is not mediated through ER-α or ER-β, and acts through another receptor system, potentially "ER-γ"
Soto et al.	1998/ISEED '98, pp. 26-27	United States	In vivo assays for hormone agonists and antagonists: the E-screen assay	E-screen assay	The E-screen detects estrogenicity chemicals in their developmental stage. It sorts out the chemicals that are presently found in the environment for further testing. It defines the estrogenic content of water courses
Kelce et al.	1998/ISEED '98, pp. 28-29	United States	Environmental antiandrogens as ED	Review	The potency of HPRE as an ER agonist and AR antagonist presents a novel mechanism whereby the detrimental effects of environmental chemicals on reproductive development and function may be amplified, particularly on the male reproductive system, which can be adversely affected through both receptor pathways
Testing and Hormone Responses					
Aoyama	1999/ISEED '99, pp. 43-44 **	Japan	Validation of reproductive and developmental toxicology studies with organochlorine pesticide	*In vitro* screening methods and *in vivo* tests (two-generation reproductive study) (rats, rabbits)	Uterotropic assay is effective. (Methoxychlor, p,p'-DDT) Toxicological significance of hormonal alterations still remains obscure
Gaido	1999/ISEED '99, pp. 46-47	United States	Characterizing chemical interaction with steroid hormone receptor	Steroid hormone receptor assay	The complexity involved in determining the mechanism of action of endocrine active chemicals that may act as agonist or antagonists through one or more hormone receptors

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Table 2 (continued from previous page)

Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Zacharewski et al.	1999/ISEED '99, pp. 48-49	United States	Molecular basis of ED – estrogen receptor interaction: a species comparison	Saturation ligand-binding analyses of GST-hERαdef (human), GST-mERαdef (mouse), etc. Computer model analysis	ERs from different species exhibit differential ligand preferences and relative binding affinities for estrogenic substances. These differences may be due to the variability in the amino acid sequence within the ER ligand binding among species
Tong et al.	1999/ISEED '99, pp. 50-51	United States	An integrated computational approach for prioritizing potential estrogenic ED	(1) Simple rejection filters or rules to exclude. (2) Qualitative activity (Structural alert, pharmacophores, classification methods). (3) Multiple QSAR model. (4) Expert system to combine information gained from (2) & (3) and other sources	Prioritizing potential estrogenic endocrine disruptors. The method reduced by some 83% the number of potential estrogens. 9,100 chemicals were identified as potential estrogens, when applied to a 58,000 chemicals identified by EPA
Mechanisms of Action					
Korach et al.	1999/ISEED '99, pp. 56-57	United States	Studying ED action and toxicology in ER Knock-out mice	Gene targeting technique	αERKO treated male and female mice showed none of the toxic effects which perinatal DES treatment WT mice showed, indicating that ERα was involved in mediating these DES effects and not Erβ
Bigsby	1999/ISEED '99, pp. 58-60	United States	Xenoestrogens: pharmacokinetics and molecular mechanism	Gas chromatography/ electron capture techniques (ovariectomized mouse)	Blood levels of organochlorine pesticide residues seen in the general population are likely to be sufficient to cause estrogenic effects and thereby pose a health concern. The mode of action of a xenobiotic may be quite distinct that considered the classic estrogen receptor mediated pathway
Fujii-Kuriyama et al.	1999/ISEED '99, pp. 61-62	Japan	Molecular mechanisms of function and regulation of Ah receptor	Gene targeting technique (mouse)	AhR and AhRR form a regulatory loop in the xenobiotic signal transduction
Kobayashi et al.	1996	Japan	Cooperative interaction between AhR.Arnt and Sp1 for the drug-inducible expression of CYP1A1 gene	In vivo transfection assays using Drosophila Schneider line 2 cells. Invitro transcription assays using baculovirus-expressed AhR, Arnt and Sp1 proteins	XRE and GC box elements are commonly found in the genes whose expressions are induced by polycyclic aromatic hydrocarbons, suggesting that the two regulatory DNA elements and their cognate trans-acting factors constitute a common mechanism for induction of a group of drug-metabolizing enzymes
Xie & Evans	1999/ISEED '99, pp. 63-64	United States	Trans-Species activation of Cytochrome P450 3A (CYP3A) gene by orphan nuclear receptor SXR	Introduction of SXR (steroid X receptor) into rat hepatocyte cultures or into the liver of transgenic mice	Human like profile of CYP3A gene activation by certain drugs such as rifampicin, establish a central role for SXR in CYP3A gene activation. Expression of an activated form of SXR and the resulting constitutive upregulation of CYP3A gene expression showed the significance of SXR in xenobiotics and steroid homeostasis

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Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Morohashi	1999/ISEED '99, pp. 65-66	Japan	Expression and function of transcription factors implicated in differentiation of the gonad	Gene encoding Gene disrupting	The mechanism underlying the gonad differentiation became uncovered at least in part. Effects of ED upon the expressions of these have been under investigation
Yuasa	1999/ISEED '99, pp. 67-70	Japan	Both spermatogenesis and neurogenesis are defective in the fyn-deficient mice: a prospect on the possible common molecular mechanism related to environmental EDs	Electron-microscopy	DES-treated testis displayed massive degeneration of spermatocytes and the delay of onset of meiosis during development. The delay of the formation of ectoplasmic specialization in the Sertoli cells at the sites of contact with spermatogenic cells was also found. Further investigations are continued
The Effect of Eds on Humans					
Silbergeld	1998/ISEED '98, pp. 30-31	United States	ED and CNS development: potential interactions between endogenous hormone and EDC	Administration of DES (female rats)	Sexual differentiation of the CNS may be a sensitive system to test effects as well as mechanisms of proposed EDCs
Darvill	1998/ISEED '98, pp. 32-33	United States	Relationships between maternal consumption of great lakes fish, heavily chlorinated PCBs, and neonatal behavior of offspring	The relationship between prenatal (cord blood) polychlorinated biphenyls (PCBs) and neonatal behavioral assessment scale (NBAS) performance in babies born to women who consumed contaminated lake Ontario fish is examined	Most heavily chlorinated PCBs would be related to impaired performance on those NBAS clusters most strongly associated with fish consumption; the Habituation, Autonomic, and Reflex clusters of the NBAS. The chlorination level of PCBs is an important factor both for exposure assessment and for determining relationships with neurobehavioral function
Brook	1998/ISEED '98, pp. 34-35	United States	Endocrine-Active compounds and human health	Review	Many of cancer particularly breast cancer, are known to be affected by endocrine-active compounds
Swan	1998/ISEED '98, pp. 36-37	United States	Variation in semen quality: assessing causes and consequence	Review	Geographic areas with depressed semen quality ate at risk of significantly impaired fertility
Mori	1998/ISEED '98, pp. 38-39	Japan	Fetal exposure to EDCs and possible effects of EDCs in the male reproductive system in Japan	Detection of EDC in human umbilical cord. Histological observation of tests, examining medicolegal data of about 5,000 Japanese men (20-39 years old).	Dioxins, PCBs, DDTs, BHC, chlordens and heavy metals (Cd, Pb, Hg) were detected in both human umbilical cords and cord blood. Bisphenol A and nonylphenols were also detected in human umbilical cords. Histological observation of tests revealed no obvious changes of spermatogenesis in Japanese men. Puberty in boys has accelerated as well as with girls in Japan
Sumiyoshi et al.	1998/ISEED '98, pp. 40-41	Japan	Surveillance of Congenital Anomalies in Japan (1972-1996)	Nationwide hospital-based monitoring for congenital malformations, which are identified after 22 gestational weeks to 7 days postpartum	One of the foremost aims of a surveillance system is to detect increase in prevalence over time, which may be due to the introduction or spread of a teratogenic exposure, whether linked to food, drugs, or domestic or outdoor environment

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Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
The Effect of EDs on Wildlife					
Guillette	1998/ISEED '98, pp. 46-49	United States	Developmental abnormalities in wildlife exposed to endocrine disrupting contaminants	Review Focal aspects of future studies	The contaminants can alter the endocrine and reproductive system of wildlife by various mechanisms other than hormone mimicry. The species variation does occur and direct linkages between wildlife and human abnormalities are unlikely
Hayes	1998/ISEED '98, pp. 50-51	United States	Model systems for examining ED in amphibian: laboratory and field-based studies	Laboratory-based comparative multi-test system to assess the effects of suspected ED on growth and development	Estrogen-dependent vitellogenin expression and oviductal growth, androgen dependent gular and thumb-pad development, thyroid hormone dependent forelimb emergence and tail resorption can be measured.
Bowerman	1998/ISEED '98, pp. 52-53	United States	Assessment of environmental ED in bald eagles of the great lakes	Aerial surveys Analysis of tissue concentrations	The effects of environmental pollutants are still evident in eagles that nest along the shorelines of the Great Lakes
Denslow	1998/ISEED '98, pp. 54-55	United States	Exposure of fish to estrogenic xenobiotics in rivers and lakes in the United States	High resolution mRNA fingerprint methods	Development of probes to follow the induction of vitellogenin, two zona radiata proteins, ZP2 and ZP3 and the estrogen receptor itself
Yokota	1998/ISEED '98, pp. 56-59	Japan	Development of testing methods for ED using Japanese medaka (Oryzias latipes)	Life cycle test Reproduction test	The effect of BPA on the early-life stage of Japanese medaka was detected only in the growth and sex differentiation. Testis-ova can be induced in Japanese medaka exposed to p-nonylphenol. The effect of E2 on reproduction was detected as the decrease of spawning eggs and the increased mortality of parent medaka themselves
Huet	1999/ISEED '99, pp. 74-76	OECD	OECD activity on endocrine disruptor test guideline development	OECD test guideline program	(i) Identify the needs and prioritise the development of new and enhanced guidelines for detection of ED. (ii) Develop an harmonized testing strategy for the screening and testing of EDC. (iii) Develop a workplan for further work in the area concerned
Bengtsson	1999/ISEED '99, pp. 79-80	Sweden	Endocrine disruption studies on fish (*in vivo*) in the Nordic countries	Overview	The speculations on a significant role of anthropogenic pollutants for reproductive disturbances in Baltic fish had encouraged the start of several research projects in the Nordic countries to study the effects and mechanism of EDs in fish
Bowmer & Borst	1999/ISEED '99, pp. 81-88	Netherlands	Environmental risk assessment for endocrine active substances-A reality	Male carp are exposed for 28d to 56d during the period of sexual differentiation	In the population where an oviduct was induced, oogenesis was never seen. Longer exposure (28 to 56d) at the two highest concentrations appeared to cause spermatogenesis to regress

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Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Ozato	1999/ISEED '99, pp. 89-92	Japan	Medaka as a model for ED substance testing	Fish test (medaka)	Medaka is useful for studying effects of ED substance on reproduction, because of a characteristic reproductive system in medaka, in which the sex is determined by XY chromosomes. New strains: d-rR-FLF strain, See-through strain
Yokota et al.	1999/ISEED '99, pp. 93-95	Japan	Development of full life-cycle test for ED using Japanese Medaka (Oryzias latipes)	Full life-cycle test (Japanese medaka, Oryzias latipes)	The full cycle test using medaka can be conducted in a shorter period (about 6 months) than other fish species and is probably capable of sufficient evaluating at least the effects of estrogenic substances
Oshima	1999/ISEED '99, pp. 96-97	Japan	Combined effects of environmental ED on reproductive success and suppression of sexual behavior in Japanese Medaka, Oryzias latipes.	Fish test (Japanese medaka, Oryzias latipes)	The combined effects of TBT+PCBs raised the possibility of suppression of sexual behavior. A reproduction test of Japanese medaka that includes a sexual behavior assay is a highly valuable method, especially for the evaluation of the combined effects of ED
Iguchi	1999/ISEED '99, pp. 114-115	Japan	Developmental effects of estrogens in animals	Outline of animal test (mouse, fish and frog)	The particular sensitivity of the developing organism to exposure to estrogenic agents in the induction of longterm changes in female reproductive organs, and persistent molecular alterations induced by the perinatal estrogenic agents
Iguchi	1998	Japan	Environmental endocrine disruptors	Review	Many chemicals released into the environment disrupt the endocrine system in wildlife and humans. More attention should be paid to abnormalities in genital organs exposed to ED during fetal and early postnatal development in wildlife and humans
Michael-Fry et al.	1999/ISEED '99, pp. 116-117	United States	Estrogenic ED impair development and reproductive behavior of zebra finches and Japanese quail	Estradiol, octylphenol and methoxychlor were injected into eggs or given orally to neonatal chicks between the ages of 5 and 11 days post-hatch, at doses from 1-1,000nmole/g body wt/day	Zebra Finches are vulnerable after hatching, being adversely affected. (brain changes in female and behavioral changes in both males and females) The disruption alters courtship and mating behaviors of birds tested in pair trials. Estrogen exposed birds failing to breed successfully
Matthiessen	1999/ISEED '99, pp. 118-119	United Kingdom	The effects of ED on fish in the United Kingdom	Review	Estrogen hormones and their synthetic analogues, which are mainly derived from human excretion, but a component consists of industrial materials (e.g. nonylphenol), are able produce a variety of sexual abnormalities.
Fossi et al.	1999/ISEED '99, pp. 120-121	Italy	Biomarkers as diagnostic and prognostic tools for wildlife risk assessment about EDs	Biomarker Potential use of BPMO (MFO) activity in skin biopsies	Statistical correlation between BPMO activity and organochlorine levels in skin biopsy specimens from males of Balaenoptera physalus

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Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Jones	1999/ISEED '99, pp. 122-123	United Kingdom	Sources, trends and foodchain transfers of persistent organic contaminants: quantifying the link between emissions, air concentrations and human exposure	Review	The relationship between emissions of PCDD/Fs and PCBs over time and how the environmental residues, distribution and foodchain transfer of these compunds will have responded to these emissions
Toxicology and Risk Assessment of EDs					
Von Saal	1998/ISEED '98, pp. 60-61	United States	Low dose effects of ED	Exposure	There are differences between adults and fetuses in plasma binding proteins and metabolism that contributes to the responsiveness of fetuses to very low opposite effects being found at high and low doses
Chahoud & Faqi	1998/ISEED '98, pp. 62-65	Germany	Effects of hormone modulating chemicals on male reproductive system after utero and lactational exposure	Exposure	TCDD and TCDD-like PCBs are capable of inducing reproductive hazard in male offspring and that most of the changes observed remain permanent
Dose-response					
Peterson et al.	1999/ISEED '99, pp. 100-101	United States	Developmental male reproductive toxicity of dioxin and di (2-ethylhexyl) phthalate in the rat	In *utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or di (2-ethylhexyl) phthalate (DEHP)	In *utero* and lactational DEHP exposure alters male reproductive system development and appears to alter sezually CNS development in rats. The pattern of effects demonstrates that (and/or its metabolites) is an antiandrogen
Lames IV	1999/ISEED '99, pp. 102-103	United States	Assessing the human health rsik from exposures to hormonally active agents	Review	Risk is the probability of harm and a function of hazard and exposure. Potency, dose and dose-response are critical elements of the hazard and dose-response assessment upon for all agents, including hormonally active ones
Ohsako et al.	1999/ISEED '99, pp. 106-107	Japan	Effects of low-dose Bisphenol-A rat spermatogenesis	Animal test (rats)	BPA, even at very low dose, could affect the spermatogenesis of the mature rat. In order to provide more useful information for the risk assessment of environmental estrogenic compund, further investigations are needed
Von Saal	1999/ISEED '99, pp. 108-111	United States	Very low doses of bisphenol A and ethinyl estradiol cause developmental abnormalities in mice	Fetal exposure to low doses of the estrogenic chemicals in mice	The risk posed by low doses of estrogenic chemicals cannot be determined from studies using much higher doses. The effects caused by bisphenol A and ethinyl estradiol are not those traditionally examined in toxicological studies

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Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Potential Effects on Human Health					
Adlercreutz et al.	1999/ISEED '99, pp. 152-153	Finland	Naturally occurring endocrine substances in the diet and in some commercial food products	ID/GC/MS/SIM	The mechanisms by which plant chemicals act on the endocrine system in man is only partially clarified. Phytoestrogens influence hormone production, gonadotropin release, many of the most important steroid biosynthetic enzymes and steroid proteinbinding. They also affect the mechanism of action of hormones and their metabolism
Needham	1999/ISEED '99, pp. 154-155	United States	Assessing exposure in epidemiological studies	Case studies	Determination of the best candidates among environmental toxicants to study further as possible initiators or promoters of ED effects
Hauser	1999/ISEED '99, pp. 156-157	United States	Resistance to thyroid hormone: implications for neurodevelopmental research on the effects of thyroid hormone disruptors	Exposure to dioxin and dioxin-like compounds (DLCs)	Exposure to DLCs during the perinatal period impairs normal TH function as well as learning, memory and attentional processes in the offspring
Yamashita	1999/ISEED '99, pp. 158-161	Japan	Thyroid disease and environmental factors; problems of risk assessment	Radiation exposure	The model of thyroid diseases related with radiation exposure can give us some hint to understand the similarity and difference betweeen environmental ED and ionizing radiation on human health
Skakkebæk et al.	1999/ISEED '99, pp. 162-165	Denmark	Recent trends in male reproductive health: evidence of increasing rates in testicular cancer and low sperm counts among men from the general population	Animal studies Experimental studies	An increase of cryptorchidism and hypospadias could be expected, in addition to a deterioration of semen quality. The role of ED or human reproduction remains to be investigated
Nakahori (Kuroki et al., 1999)	1999/ISEED '99, pp. 170-171	Japan	Spermatogenic ability is different among males in different chromosome lineage	Statistical observation (Japanese men)	The spermatogenic ability differes among males. The response to ED differes among group of males, in which the azoospermia is related to the certain haplotype
Basic Biology					
Nagahama	1999/ISEED '99, pp. 174-175	Japan	Introduction	Estrogen treatment Tilapia (Orechromis niloticus)	Male tilapia can be sex reversed to phenotypic females by estrogen treatment during gonadal sex differentiation. ERα and β exhibit different expression paterns suggesting different roles of Erα and ERβ in estrogen action on gonadal sex differentiation
Oehlmann et al.	1999/ISEED '99, pp. 176-178	Germany	The mechanism of TBT-induced imposex in marine neogastropods	TBT pollution	Imposex is a form of ED caused by elevated testosterone titres that masculinize TBT-exposed females. An exposure to a specific steroidal inhibitor of the aromatase enzyme resulted in an increase of imposex intensities in the female of *N. lapillus* and H. reticulata

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Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Thomas	1999/ISEED '99, pp. 179-180	United States	Xenobiotic chemical interference with nongenomic steroid actions	Bioassays	Several estrogenic chemicals were found to bind to the ovarian maturation-inducing steroid (20 β-S) membrane receptor in spotted seatrout and antagonize 20 β-S-induced meiotic maturation of oocytes in this species
Blumberg et al.	1999/ISEED '99, pp. 181-182	United States	Deformed frogs and environmental retinoids	Methoprene treatment of developing frog embryos	Multiple fractions that can activate to active retionoid signaling pathways and to perturb frog development, were found in the water from an effected Minnesota site
Crews & Willingham	1999/ISEED '99, pp. 183-186	United States	The slider turtle: an animal model for the study of low doses and mixtures of EDC	Exposure of mixed EDC	EDCs not only exert effects during embryonic development that extend beyond birth. The alterations in sex steroid hormone levels observed in animals from contaminated areas may result from EDC-induced alterations in the neuroendocrine axis controlling gonadal sex steroid hormone production
Ottinger	1999/ISEED '99, pp. 187-188	United States	Behavioral implications of contaminant exposure: neuroendocrine mechanisms	Exposure of EDC	Distinct effects of suspected EDCs on behavioral responses in adults exposed during embryonic development. Behavioral effects might occur in field populations with sufficient exposure
Gore et al.	1999/ISEED '99, pp. 189-190	United States	Effects of environmental toxicants on GnRH gene expression in hypothalamic neurons	Exposure to urban environmental toxicants during development	Environmental toxicants can exert their effects directly on hypothalamic neurons
Pesticide					
Obana et al.	1997	Japan	Determination of organophosphorus pesticides in foods using an accelerated solvent extraction system	Residual organophosphorus pesticides in foods were determined by Accelerated solvent extraction (ASE), gel permeation chromatography and GC-FPD	ASE can be used to extract residues of organophosphorus pesticides in foods
Okihashi et al.	1998	Japan	Determination of N-methylcarbamate pesticides in foods using an accelerated solvent extraction with a mini-column cleanup	Residual N-methylcarbamate pesticides in foods were determined by ASE and HPLC with post-column fluorescence	ASE can be used to extract residues of N-methylcarbamate pesticides in foods
Activities in Japan					
Ohi	1999/ISEED '99, pp. 126-129	Japan	Social Medicine and ED		The countermeasure appears to be based on the fact that the primary rate of ED uptake is through diet: Information regarding the ED contamination of food must be disclosed without failure so that consumers can make choice based on the information

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Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Shiraishi	1999/ISEED '99, pp. 130-132	Japan	Some aspects in chemical analysis of EDs	GC/MS ELISA	Sensitivity of ELISA is sufficient, but accuracy was found to be not enough. Two methods (1) GC/MS after methyl derivation and (2) Negative Chemical Ionization (NCI)-GC/MS after pentafluorobenzyl derivation, with detection limit near 0.1ng/L has been proposed. Developing more efficient and sensitive simultaneous determination method must be continued, in addition developing bioassay system is necessary to prevent exposure from unkown ED
Umeda	1999/ISEED '99, pp. 134-135	Japan	Sperm quality of the Japanese general population	Analysis of 270 Japanese men's semen profile	The mean value are almost the same in the three groups, Tokyo area, Kyushu area and Matsuyama area, however, the median of the 20's was smaller than the other groups. Further studies are needed to estimate the changes in sperm quality of Japanese men
Tsutumi	1999/ISEED '99, pp. 136-139	Japan	Low dose effects of ED on preimplantation embryo development	Exposure of TCDD (mice) GM-MS (human)	Lower concentration of both TCDD and BPA, not dose dependent manner, exert stimulatory or inhibitory effects on preimplantation embryo development. The contamination of the follicular fluid with PCDDs, PCDFs and BPA indicate that the reproductive organs have been exposed to these substances, and that the germ cells and early embryos may be affected, as those substances can adversely affect mammalian fetal development
Yoshizato	1999/ISEED '99, pp. 140-141	Japan	Detection of ED substances by transgenic frogs	Transgenic Xenopus laevis into which gene constructs containing a 5'-upstream promotor region of TH receptor β (TRβ) and an enhanced green fluorescent protein (EGFP) gene as a reporter gene	The tadpoles with transgene containing promoter of TR_ gene can give us visible signals when they are given substances, which react with the promoters. These transgenic animals might be useful to detect environmen tal substances, which disturb a step of the TH-related endocrine system. The transgenic Xenopus laevis into which gene constructs containing promoters of mouse metallothionein and gene of EGFP was also made
Hashimoto	1999/ISEED '99, pp. 142-145	Japan	Elevated serum vitellogenin and gonadal abnormalities in wild male flounder from Tokyo Bay, Japan	Luminometric immunoassay (flounder)	The VTG levels in male flounder in Tokyo Bay (n = 130) were significantly higher compared to those of the reference fish from Hokkaido (n = 62)
Nagao	1999/ISEED '99, pp. 146-147	Japan	Effects of prenatal exposure to styrene dimers and trimers on reproduction in rats	Animal test (rats)	Stylene dimer and trimer (SDT) administered as described in this study at dose levels as high as 1.0mg/kg (1,000 times of estimated daily intakes) did not demonstrate any reproductive toxicity in dams and offspring

(to be continued on next page)

Table 2 (continued from previous page)

Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Tokuchi et al.	1999	Japan	Expression of protein tyrosine phosphatase PTP-RL10 and its isoform in the mouse testis	Tests of wildtype mice and W/W(v) mutant mice that lack germ cells were analyzed by northern blotting, insitu hybridization histochemistry and reverse transcriptase-polymerase chain reaction for the expression of PTP-RL10, its isoform PTP-RL10b and c-src	PTP-RL10 and its isoform are expressed in the Sertoli cells and are suggested to play roles in spermatogenesis by interacting with c-Src and/or other proteins
Takeda & Yoshida	1999/ISEED '99, pp. 148-149	Japan	Effect of diesel exhaust (DE) on the murine male reproductive system and evaluation of its ED activity	Male mice at 6 weeks of age were exposed to DE for 12 hours a day for 6 months	DE may potentially influence spermatogenesis in men. Certain substance(s) in DE, but not dibenzodioxins, remarkably suppress the expression of androgen and estrogen receptor mRNA of cultured testis cells in vitro. Further studies are necessary to define which classes of compounds are responsible for the changes in testicular function
Yoshida et al.	1999	Japan	Exposure to diesel exhaust affects the male reproductive system of mice	Observation of Leydig cells of mice exposed to diesel exhaust (0.3mg diesel exhaust particle (DEP)/m3 through the airway, 12h daily, up to 6 months)	Ultrastructural changes and reduction in LH receptor mRNA expression was observed at a concentration of 1mg DEP/m3. Daily sperm production per gram of tests dose dependently decreased. A no-observed-adverse-effect level (NOAEL) was observed with approximately 30microg DEP/m3
Kawamura	1999	Japan	EDC in food container and utensils	GC/MS/SCAN	Endocrine disruptor chemicals were detected in food contact plastics, although the affection rate was not clear
Jiang et al.	1998	Japan	Eel (*Anguilla japonica*) testis 11beta-hydroxylase gene is expressed in interrenal tissue and its product lacks aldosterone synthesizing activity	Gene expression Northern blot analysis	No hybridization signal was apparent using RNA extracted from brain, spleen, heart, muscle or testis. Immunohistochemistry using an antiserum against P450 (11beta) also revealed strong immunostaining in interrenal cells
Chang et al.	1997	Japan	Isolation and characterization of the cDNA encoding the tilapia cytochrome P450 aromatase (P450arom)	Northern blots Western blots	The capacity of tilapia ovarian follicles to synthesize estradiol-17 beta is closely related to the contents of $P450_{arom}$ mRNA and protein within them
Takeyoshi et al.	2000	Japan	Changes in serum alpha 2u-globulin (AUG) levels in male rats given DES and applicability to a screening test for endocrine-disrupting chemicals	AUG-based screening test (rats)	An AUG-based screening test for estrogenic chemicals may be useful owing to its applicability to conventional toxicity studies and an apparently higher sensitivity of this parameter compared to organ weight change or histology of testis in intact male rats and applicability to conventional toxicity studies
Nishikawa et al.	1999	Japan	New screening methods for chemicals with hormonal activities using interaction of nuclear hormone receptor with coactivator	Hybrid assay	The measurement of interaction between steroid hormone receptor and coactivator serves as a useful tool for identifying chemicals that interact with steroid receptors

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Table 2 (continued from previous page)

Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Ohkura et al.	1999b	Japan	An isoform of Nurr1 functions as a negative inhibitor of the NGFI-B family signaling	DNA hybridization Isolation of Nurr2 from a mouse MC3T3-E1 cell cDNA library	The C-terminal truncated isoform, Nurr2, may act as a negative regulator of the NGFI-B family signaling
Nagasaki et al.	1999a	Japan	Identification of a novel gene, DAM1, amplified at chromosome 1p13.3-21 region in human breast cancer cell lines	Southern blot analysis	The DAM1 gene is a novel gene up-regulated by amplification in human breast cell lines
Ikeda et al.	1999	Japan	Anophthalmia in litters of female rats treated with the food-derived carcinogen, 2-amino-1-methyl-6-phenylimidazol [4,5-b]pyridine (PhIP)	Risk assesment study with 2-generation exposure to PhIP (rats)	PhIP is capable of causing anophthalmia in rats when administered during the gestational period
Nagao et al.	2000b	Japan	Possible mechanism of congenital malformations induced by exposure of mouse preimplantation embryos to mitomycin C	ICR mice were treated intraperiotoneally with mitomycin C at 5mg/kg on day 3 of gestation	An increase in number of degenerated cells within blastocysts results in preimplantation loss, and both maternal and embryonic hypoxia during major organogenesis results in postimplantation loss and congenital fetal malformations
Nagao et al.	1999a	Japan	Developmental toxicity of the topoisomerase inhibitor, etoposide, in rabbits after intravenous administration	Intravenous doses during early organogenesis, using pregnant rabbits	Anatomical defects (skeletal malformation or variation) in rabbits were induced by intravenous etoposide treatment during early organogenesis and that they occured in the present of maternal toxicity
Nagao et al.	1999b	Japan	Reproductive function in rats exposed neonatally to bisphenol A and estradiol benzoate	Subcutaneous treatment with 300microg/g bisphenol A or 2microg/g estradiol benzoate from postnatal day 1 to 5 (rats)	Neonatal exposure to estradiol benzoate affects reproductive function in male and female rats, and treatment with bisphenol A at afairly high dose was ineffective if given postnatally to male and female rats
Ohta et al.	2000	Japan	Postnatal behavior in hatano high- and low-avoidance rats following prenatal exposure to low-dose methylazoxymethanol	Behavioral test in rats exposed prenatally to methylazoxymethanol (MAM) Locomotor activity Learning activity	Prenatal exposure to low doses of MAM may alter postnatal behavior and endocrine response of the offspring, although to a differing degree in high-avoidance animals and low-avoidance animals. Suggestion: behaviorally selected strains are sensitive to neurobehavioral teratogens such as MAM
Tamura et al.	1999	Japan	Time course observation of thyroid proliferative lesions and serum levels of related hormones in rats treated with kojic acid after DHPN initiation	Examination of time course changes in thyroid proliferative lesions as well as related hormone level in the blood of male F344 rats. N-bis (2-hydroxypropyl) nitrosamine	Thyroid proliferative lesion were induced by kojic acid administration due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis, resulting from depression of serum T3 and T4
Ema et al.	2000	Japan	Effects of dibutylphtalate on reproducctive function in pregnant and pseudopregnant rats	Animal test (rats)	Early embryonic loss due to DBP may be mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function

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Table 2 (continued from previous page)

Authors	Year/Reference	Country	Topics	Methods	Outcome/Results
Ema et al.	1999a	Japan	Suppression of uterine decidualization as a cause of implantation failure induced by triphenyltin chloride in rats	Animal test (rats)	Implantation failure due to TPTC1 may be mediated via the suppression of uterine decidualization and correlated with the reduction inserum progesterone levels
Ema et al.	1999b	Japan	Adverse effects of diphenyltin dichloride on initiation and maintenance of pregnancy in rats	Animal test (rats)	DPTC1 during early pregnancy causes embryonic loss and DPTC1 has greater effects on reproduction when administered during earlier than later stages of blastogenesis
Suzuki et al.	1999b	Japan	Preparation of specific antisera to 15alpha-hydroxyestrogens	Enzyme immunoassay	The specificity of antisera elicited against bovine serum albumin conjugates was checked and proved to be satisfactory in terms of cross-reactivities to related compound
Nakagomi et al.	1999a	Japan	Preparation of specific antisera to 15alpha-hydroxyestrogen 15-N-acetylglucosaminides	Synthesization and conjugation with bovine serum albumin	Demonstration of the first time that the conjugation of N-acetylglucosamine to E4 occurs at the C-15alpha position
Nakagomi et al.	1999b	Japan	Enzyme immunoassay for measurement of 17alpha-estradiol 17-N-acetylglucosaminide in rabbit urine	Enzyme immunoassay	17alphaE2 17NAG was mainly excreted as a double conjugate. Its level varies during pregnancy
Nakata et al.	1999	Japan	Receptor database	Database Internet	Receptor information: structure (1,2,3-dimensional), function, binding information, gene, transcription, etc.
Nakano et al.	1998	Japan	Endocrine disruptors structure database	Database	CAS registry number, synonyms Structure information, etc.
Kaminuma et al.	2000	Japan	Binding affinity database	Database	Experimental data for exogenous chemicals and bio-molecules

*ISEED '98 = International Symposium on Environmental Endocrine Disruptors '98.
** ISEED '99 = International Symposium on Environmental Endocrine Disruptors '99.

Enzyme Induction Experimental results were included in BADB. Figure 1 shows the relative binding affinity between human estrogen receptor α/β and ligands, which were picked up from BADB.

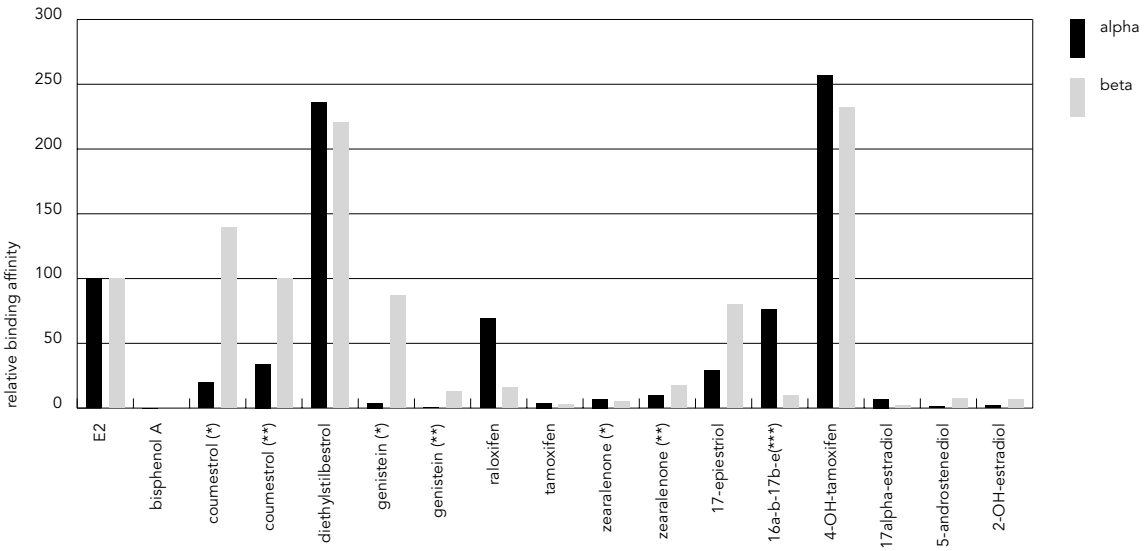
RDB included various receptor information, for instance structural information (amino acid sequences, secondary structure prediction, three dimensional structure image), functional information (DNA binding sites, ligand binding sites, etc.), genetic information (DNA sequences, gene information), the cell signaling networks information and transcription information. At present (June 2000), RDB contains 1,576 receptor proteins. The three-dimensional structure information was included in 121 receptor proteins. The DNA binding site and ligand binding site information were included in 206 and 130 receptor proteins, respectively. The data for transmembrane region was included in 1,070 receptor proteins. Recent versions of RDB included both information of EDSD and BADB, and much more. Using the table *Steroid Hormone Receptor and Possible Endocrine Disruptor* in the top page of RDB <http://impact.nihs.go.jp/RDB.html>, users can refer to a steroid hormone receptor and possible endocrine disrupters (Figure 2).

Global Information Network on Chemicals

In June 1992, the United Nations Conference on Environment and Development Collection (UNCED) was held in Rio de Janeiro, Brazil. Chapter 19 of Agenda 21 of UNCED (UN, 1992) calls on communities "*to promote intensified exchanges of information on chemical safety, use and emissions among all involved parties*". It recommends that governments and relevant international organizations with the co-operation of industry should strengthen national institutions responsible for information exchange on toxic chemicals and strengthen international institutions and networks responsible for information exchange on toxic chemicals. Within the frame of the Inter-Organization Program for the Sound Management of Chemicals (IOMC), World Health Organization (WHO), International Labour Organization (ILO), United Nations Environmental Programs (UNEP) and Organi-

Figure 1

Binding affinities between human estrogen receptor and ligands



Alpha: estrogen receptor alpha; Beta: estrogen receptor beta;
* Nuclear extract; ** Whole cell extract; *** 16alpha-bromo-17beta-estradiol

zation for Economic Cooperation and Development (OECD), with the support of the NIHS Japan initiated a project to establish a Global Information Network on Chemicals (GINC). The aim of GINC project is to provide networking arrangements for linking and better access by various users to existing international databases and documents on chemicals, as well as databases and chemical information systems available, or to be developed in each country.

Since the Internet has been launched and the WWW has become popular, the idea spread widely. GINC Home Page <http://www.nihs.go.jp/GINC/index.html> was designed for the navigation to a wide range of WWW pages and databases in order to find useful information related to chemical safety: regulations, chemical names and synonyms, physico-chemical properties, toxicological data, protection for workers, environmental exposures, assessments and regulations. The United Nations organizations, the OECD, Environmental Protection Agency (EPA, United States), National Institute of Environmental Health Science (NIEHS, United States), and other Center of Excellences (COEs) have provided this information.

For the first GINC meeting at Tokyo 1994, only Korea and Japan were represented from Asia. Since then, attendance from China, Indonesia, Korea, Philippine, Singapore, Sri Lanka, Thailand and Vietnam gathered with the members from International Program on Chemical Safety/WHO (IPCS/WHO), International Register of Potentially Toxic Chemicals (IRPTC/UNEP), International Occupational Safety and Health Information Centre (CIS/ILO), OECD, EPA, NIEHS and Japan in GINC Asia Meeting. The corresponding organizations opened their Web Page for information exchange. These Home Pages and the GINC Asia page <http://www.nihs.go.jp/GINC/collabo.html> were connected reciprocally.

Discussion

EPA formed the Endocrine Disruptor Screening and Testing Committee (EDSTAC) in 1996, and provided the reports EDSTAC via WWW. Under the legislation, some 80,000 existing chemicals and new chemicals were undergoing various screens and/or tests for their potential estrogenicity, as well as other hormonal activities. The integrated computational approach was reported to set priorities for these chemicals for experimental screening and testing (Tong et al., 1999). The OECD endocrine disruptor activity was initiated in 1996. The focus of the activity was to provide information on national and regional activities concerning endocrine disruptors, develop appropriate Test Guidelines and harmonize risk assessment approaches (Huet, 1999).

In the International Symposium on Environmental Endocrine Disruptors at Kobe 1999, many speakers from Denmark, Finland, Germany, Italy, Japan, Netherlands, OECD, Sweden, United Kingdom and United Station reported their works, and had heated discussion with the participants (Table 2). We expect these activities will lead to solutions for the endocrine disruptor problems.

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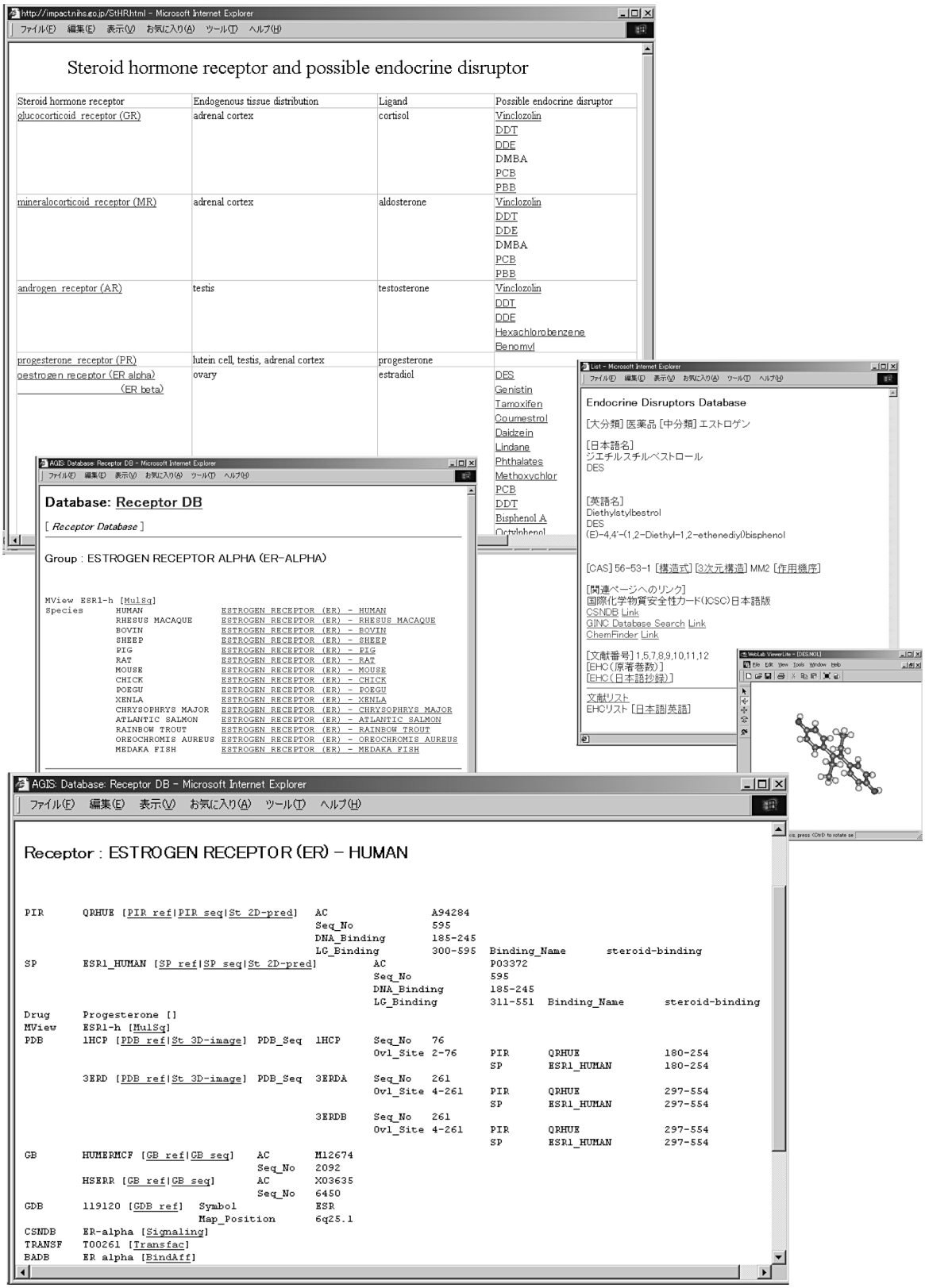
Figure 2

Steroid hormone receptor data and possible endocrine disruptor.



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