21st Century Chemistry and the *Promiscuity* of the Sex Hormone Receptors: An Overview

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Introduction

Modern chemistry has brought us many benefits from pharmaceuticals to advanced materials. With these advances comes a responsibility to balance the benefits against undesirable environmental effects. This balance is critical when chemicals interfere with the most fundamental biological process – reproduction. Recently, interference of this type was demonstrated when the population of fathead minnows in an isolated Canadian lake was brought to near extinction in just 7 years by exposure to parts per billion concentrations of 17α-ethynylestradiol (**6**), the synthetic estrogen used in birth control pills.[1] The reproductive capacity of both male and female fish was affected by the exposure. A similar outcome is possible for humans on exposure to hormonally active chemicals at inappropriate times.

The sex hormones, the estrogens and androgens, are chemical messengers in the human endocrine system. They are small molecules that bind to specific receptor proteins in the first step of a complex and tightly regulated sequence of events that controls growth, sexual development, and reproduction (Fig. 1). There are three endogenous or naturally occurring estrogens **1-3** (17β-estradiol, estriol and estrone) and two endogenous androgens **4** and **5** (testosterone and 5α-dihydrotestosterone). The estrogens bind to two distinct estrogen receptors, ERα and ERβ, and the androgens bind to a single androgen receptor, AR. The estrogens and androgens, and their receptors, are present in both males and females but in different amounts (Table 1). ERα is primarily responsible for the development of female sex organs and secondary feminine characteristics, such as enlarged breasts. AR is responsible for male sex organ development and secondary masculine characteristics, such as facial hair and deepening of the voice. There are very few locations in which both ERα and ERβ are expressed in significant amounts, and, at those locations, the two receptors are found in different cell types. ERα appears to be involved in activating cellular functions, including cell division, while ERβ acts in suppressing these functions. The AR is widely distributed and appears in significant amounts in nearly all tissues in which the ERs are found.[2]

Although the sex hormone receptors have evolved to be highly specific in recognizing and binding with their intended endogenous ligands, they are apparently easily fooled by 21st century chemicals. Many modern chemicals are able to bind with the sex hormone receptors and either initiate, or block, the same chain of events that endogenous hormones control. This results either in amplification or cancellation of natural hormone signals at inappropriate times. These so-called endocrine disrupting compounds

Table 1. Distribution and relative abundance of the human estrogen receptors – see ref. 2

ERα Predominant	ERβ Predominant	Both ERα and ERβ
Uterus	Prostate	Mammary gland
Vagina	Testis	Bone
Testis	Leydig cells in adult	
Leydig cells in fetus germ cells	Ovary	
Sertoli cells in adult spermatozoa	Thyroid	
Liver	Skin	
Kidney	Bladder	
	Gastrointestinal tract	
	Salivary glands	
	Heart	
	Blood vessels	
	Certain neurons in central and peripheral nervous system	
	Immune system	

(EDCs) are present as contaminants in our food and water, from pharmaceuticals (**6**, **10**) and from plastics (**7-10**) and detergents (**8**, **9**). They are used in personal care products as preservatives (**13**), as ultraviolet light stabilizers (**17-20**) and as active ingredients in sunscreens (**17-21**), and antibacterial hand soap and toothpaste (**11**). Some EDCs (**10**, **12**, **14-16**) are produced in the body by the metabolism of other chemicals, while others (**22-24**) occur naturally in foods such as soy. EDCs have many different chemical structures, but it is the combination of H-bonding functionality located at opposite ends of the molecule, *e.g.* the C3 and C17 hydroxyl groups of **1**, and the hydrophobic nature of the intervening molecular framework that is necessary for binding with the receptor.

At levels of hormonal activity, EDCs are not toxic in the classical sense in that exposure is not fatal, but they do have profound effects on both individuals and populations as shown by the declining populations of bald eagles in Florida (1952) and of seals in northern Europe (1988). In 1992, declining sperm count was the first human effect attributed to EDC exposure. These, and other observations, were finally connected and brought to the public stage in the 1996 book *Our Stolen Future*[3] that is to endocrine disrupting chemicals what Rachel Carson's *Silent Spring* was to pesticides in the 1960s.

EDCs have been implicated in a variety of medical problems in humans including uterine fibroids and ovarian

tumours,[4,5] hormone related cancers,[6] deformities of the male genitalia,[7] precocious puberty in girls,[8] declining male and female fertility,[9] obesity,[10] and developmental disabilities and changes in sexual behaviour.[11] These adverse effects arise not only from exposure as an adult but also from relatively small doses during specific windows of vulnerability.[4] One critical period appears to be during early embryonic and fetal development, when cells are rapidly differentiating and tissues are growing quickly. The fetus is protected from high levels of natural hormones by the placenta, a protection assumed to extend to foreign chemicals. However, EDCs are able to cross the placenta.[12-15] Developmental exposures are particularly difficult to monitor and quantify since the outcomes may not be observed until many years later.

Hormone Action and Interference

Two factors are important in the control of cellular response to hormones, the availability of free hormones at the sites of the hormone receptors, and the geometry of the receptor binding pocket. Interference with either of these can result in inappropriate hormone action.

Sex hormone concentrations in the blood are tightly controlled to ensure that they act only at both the desired time and place in the body. The hormones are hydrophobic and have limited solubility in blood. To fulfil their role as chemical messengers, circulating hormones are bound to plasma-steroid-binding proteins for transport from the site of production to where they are needed. The human sex-hormone-binding globulin (hSHBG) is the main specific transport system for sex hormones. Estrogens and androgens bind to different sites on hSHBG.[16] Since only free hormones are able to bind with their receptors, transport-binding proteins play an important role in controlling the concentrations of free hormones in the body. Certain EDCs, *e.g.* alkylphenols **7-9** but not phthalates **10**, are able to bind hSHBG in a reversible and competitive way and displace the endogenous hormones, resulting in an increase in blood concentrations of the free hormones.[16,17] The binding of EDCs with hSHBG is generally weak. Their affinity constants are 10^3-10^4 times lower than those of the endogenous hormones with the effect that a larger fraction of the total EDC concentration is available to interact with the receptors. There is also evidence that EDCs act additively or synergistically. Even though the concentration of an individual compound may be low, humans are exposed to a mixture of EDCs, and the sum of the individual concentrations can be high enough to induce an effect.[18,19]

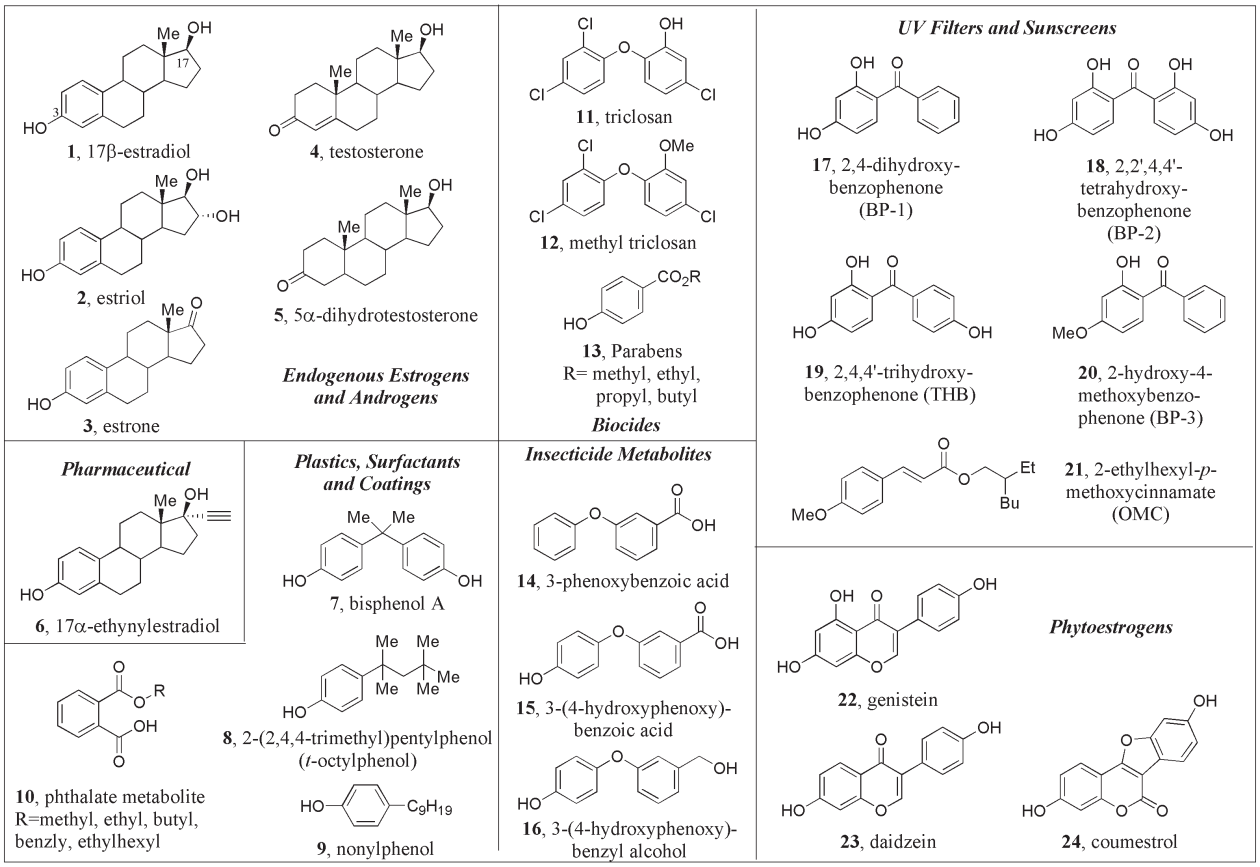
The estrogen receptor binding pocket (450 Å^3) is large in comparison to its endogenous ligand, **1** (17β-estradiol: 245 Å^3).[20] and it is very hydrophobic, as expected for the accommodation of **1**. The two main ligand-anchoring mechanisms in ER are i) a three-way H-bonding bridge between the phenolic H of estrogen and two amino acids and a water molecule at one end of the pocket, and ii) an H-bond between the 17β-OH of the estrogen and a single amino acid at the other end of the pocket. The three-way H-bonding arrangement works with hydrophobic residues in the region to form a clamp that holds the estrogen aryl ring in place so that the H-bonding at the opposite end of the ligand further secures the ligand in the pocket. The rigidity of ligand **1** requires the precise interaction of these residues for its tight binding. The aryl clamp also aids in discriminating between estrogens and androgens. The androgen receptor is similarly oversized for its endogenous ligands and a similar water-mediated H-bonding network serves to anchor the androgen ligands via the ketone group.[21]

On binding their endogenous hormone ligands, the ERs and AR undergo a conformational change that enables them to dimerize, recruit co-factor proteins, and bind to DNA to initiate transcription of their target genes. EDCs that mimic endogenous hormones in this process are called agonists and those that disrupt this process are called antagonists. A single EDC can have any combination of agonistic or antagonistic behaviour with the ERs and AR, as well as a range of efficiencies with which they are able to initiate transcription of the target genes. For example, the isoflavone genistein (**22**) has a binding affinity for ERβ *ca*. ~30-fold higher than for ERα, but only a 4- 5-fold increase in its ability to express an ERβ-selective gene over an ERα-selective one. However, it is less efficient in expressing an ERβ-selective gene over an ERα-selective one.[22] Isoflavone **22** is described as a selective ERβ modulator, a full agonist for ERα and a partial agonist for ERβ. It also illustrates the fact that strength of receptor binding does not necessarily reflect a ligand's ability to initiate gene transcription. This selective EDC modulation is a manifestation of the extent that the conformational change, which occurs in the receptors on ligand binding, influences the efficiency of receptor dimerization and continues along the transcriptional activation path. This differential receptor activation and deactivation is dependent upon both the receptor type and the specific EDC. This complex ligand-receptor interaction, coupled with the differential distribution of receptors in male and female tissue (Table 1), results in effects that are difficult to predict. This is because they can either compete or act synergistically, depending on the sex of the individual and the range and relative amounts of EDCs to which the individual is exposed.

The *promiscuity* of sex hormone receptors is manifest in their inability to distinguish between their endogenous ligands and EDCs. It is possible that the rigidity and similarity of molecular structure of the estrogen and androgens have driven the receptors to develop these highly specific H-bonding networks to discriminate between the intended hormone and other hormones and, at the same time, retain their binding pocket size. This large, somewhat plastic binding pocket, coupled with their relative flexibility, enables the EDCs to bind with the receptor, although with much lower affinity compared to the endogenous ligands. EDCs are able to adopt conformations in the binding pocket that may not necessarily be the low energy ones in free solution. The H-bonding and hydrophobic interactions with the surfaces of the pocket provide additional stability to permit EDCs to bind with differing affinities.

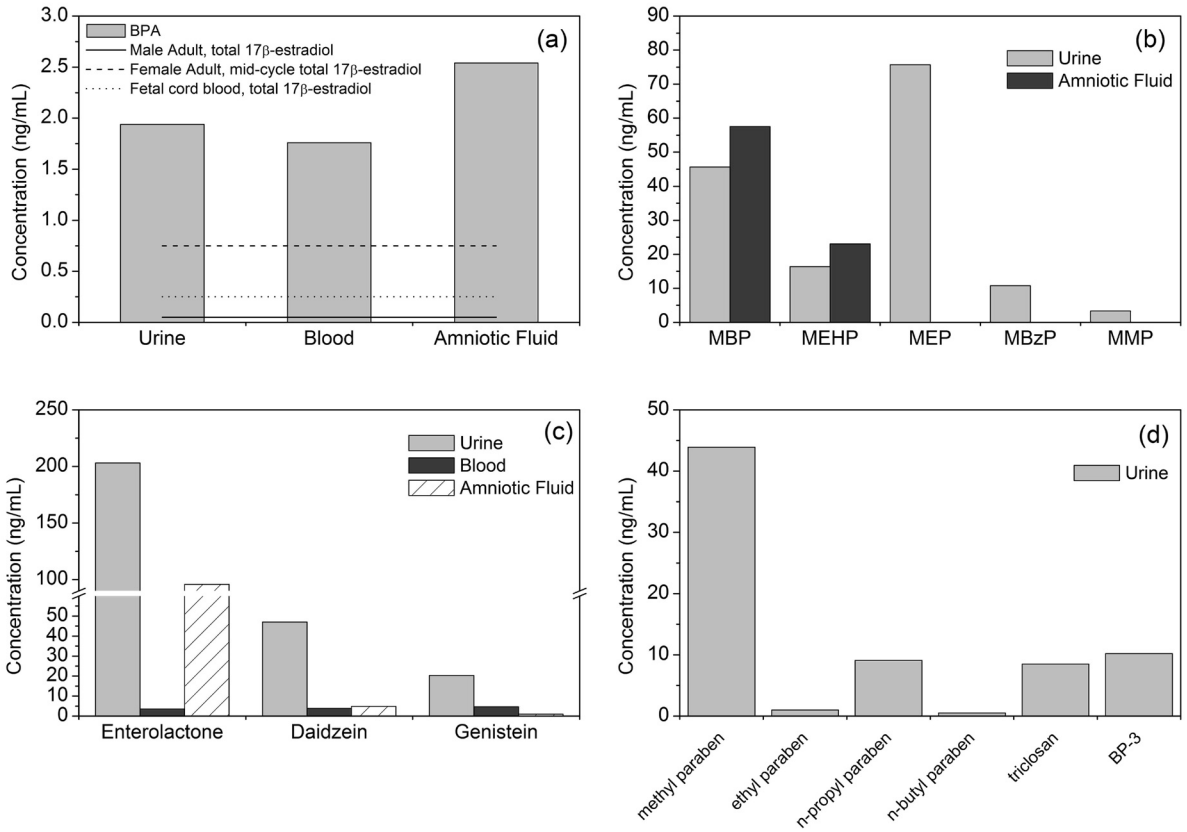
Fetal Exposure

Several EDCs have been detected in amniotic fluid[13-15,23] and in a variety of other human biological fluids and tissues that include blood (serum or plasma),[23-25] umbilical cord blood,[23,24] urine,[13,26] semen,[27] ovarian follicular fluid,[23] breast milk,[28] and placental tissue.[24] These studies have each focused on a single compound, *e.g.* bisphenol A (**7**), or a family of compounds, *e.g.* selected phthalate metabolites **10** or the parabens **13**. However, they have not looked at mixtures of EDCs arising from different sources. Selected results have been combined and are presented in Fig. 2. For reference, total serum levels of **1** (17β-estradiol) for adult males and females and for umbilical cord blood are also included. As Fig. 2 shows, the measured levels of individual EDCs are much higher than endogenous hormones in the fetus, and in adult males and females. Clearly, the mechanisms of the placenta that protect the fetus from maternal hormones are not effective with EDCs.



Compounds	Source
1-3	Endogenous estrogens
4, 5	Endogenous androgens
6	Synthetic estrogen used in oral contraceptive pill
7	In polycarbonate plastic food containers, epoxy linings of food cans, epoxy dental fillings
8, 9	Surfactants, detergents, plasticizer in some polystyrene products
10	Primary metabolite of phthalate diesters that are plasticizers in a variety of plastics, in personal care products to maintain suspensions and in making enteric coatings for medications
11, 12	Biocides. Used in various personal care products (waterless hand sanitizers, soaps, deodorants, toothpastes, shaving creams, mouth washes), medical wash products for skin infections. Infused in consumer products e.g. kitchen utensils, toys, bedding, socks, and trash bags.
13	Preservatives - bactericides and fungicides: In personal care products (shampoos, commercial moisturizers, shaving gels, personal lubricants, topical pharmaceuticals, spray tanning solution and toothpaste. Also used as food additives
14-16	Metabolites of permethrin and cypermethrin are active components in domestic aerosol insecticides
17-20	Hydroxybenzophenones: UV filters used in sunscreen products, added to personal care products and their packaging to protect the product from UV degradation
21	UV filter used in sunscreen products
22-24	Phytoestrogens: Occur naturally in nuts, oilseeds, legumes, soybeans, Brussels sprouts, alfalfa, spinach and clover

Fig. 1. Endogenous hormones, selected endocrine disrupting compounds and their sources.



a) Bisphenol A levels in urine, blood, and amniotic fluid compared with total blood levels of 17β-estradiol (**1**) in adult males, females and umbilical cord blood; b) phthalate ester metabolites in urine and amniotic fluid: MBP = monobutyl phthalate; MEHP = monoethylhexyl phthalate; MEP = monoethyl phthalate; MBzP = monobenzyl phthalate; MMP = monomethyl phthalate; c) phytoestrogens in urine, blood, and amniotic fluid; d) EDCs from personal care products in urine.

Fig. 2. Comparison of EDC levels in human biological fluids. Personal care products in urine: parabens (**13**), triclosan (**11**), BP-3 (**20**).

EDC exposure is believed to be most critical during the period of human fetal development when sexual differentiation occurs – within the first 20 weeks of gestation. Around the 11th week, the fetus begins to produce urine that enters the amniotic fluid. The amniotic fluid is swallowed by the fetus and absorbed by the gastrointestinal and respiratory tracts. EDCs in the amniotic fluid are present from maternal exposure and are ingested by the fetus, resulting in systemic exposure. Thus, amniotic fluid obtained between 16 and 20 weeks gestation is seen as a valid representation of fetal exposure during the period of sexual differentiation.[15]

Challenges in Attribution of Adverse Effects

EDCs do not behave like traditional toxic compounds and, therefore, are not amenable to the conventional environmental risk assessment framework. This framework was established by the US National Research Council in the early 1980s and has been almost universally adopted.[29] It includes the following steps i) hazard identification, ii) dose-response assessment, iii) exposure assessment, iv) risk characterization, and has several shortcomings:[30]

i) The target of the risk assessment is the adult. It does not consider effects on children or the fetus.

ii) Risk is assessed for each chemical individually and not admixed with other chemicals.

iii) The framework is designed to detect high dose effects of chemicals over short time periods.

iv) The framework does not detect the effects of long-term, low level exposure to multiple chemicals.

All of these shortcomings are critical in the risk assessment of EDCs.

In the last two decades, greater focus has been placed on vulnerable populations in risk assessments. These *vulnerable* populations have generally included children, the elderly, and other *susceptible* individuals who, by genetic predisposition or some illness or disease state, are more likely to be affected than the normal healthy individual. The fetus has yet to be included in the vulnerable population category.[31] At the same time, the importance of mixtures of chemicals in risk assessment was recognized and the conceptual challenges in dealing with combination effects have been articulated by Kortenkamp and co-workers over the last decade.[18,32]

Mixtures and synergistic effects

Characterizing how EDCs act together requires new models to quantify effectively both the relative potencies of the individual components and their relative amounts in the mixture. The concept of potency is somewhat ambiguous as it is usually defined with reference to a particular

end point or function (Table 2). Potency depends on the identity of the EDC, the receptor to which it binds, the degree to which the EDC is an agonist or antagonist for the receptor, the relative distribution of the receptors in the target tissue, and the sex of the individual. Currently, potency is operationally defined by the choice of assay (Table 2) and that choice will result in a selection of specific EDCs for ranking in different orders of relative potency.

The relative proportion of chemicals in a mixture is more straightforward to determine. However, if the observed effect is due to a metabolite of a given chemical and not to the chemical itself, then this knowledge is critical in deciding what needs to be quantified.

The degree of agonism or antagonism an EDC expresses cannot be determined solely by measuring binding affinity to the receptor, as shown by the example of **22** (genistein) given above. It can only be characterized by an assay that includes some type of transcriptional activation. In a mathematical context, agonism may be thought of as having a positive sign, antagonism having a negative sign, and selective modulator having a sign dependent on the receptor subtype. This now allows for two chemicals in a mixture, with one able to mathematically cancel the effect of the other, when present in the correct proportion. Whether this happens *in vivo* has yet to be established.

Low Dose and End Point

The definition of low-dose is challenging. For evaluating EDCs, the clinical thresholds in endocrinology can provide some guidance for evaluating toxicological thresholds. The shift in balance of estrogenic and androgenic effects may be small in a female of reproductive age who is already experiencing monthly blood free 17β-estradiol (1) concentration[33] swings of from 0.3 pg/mL to greater than 4 pg/mL and up to 1000 pg/mL during pregnancy, but in an adult male or pre-pubertal males and females (<0.9 pg/mL) the shift may be significant. This shift is also likely to be significant in a developing fetus, especially during sexual differentiation, when concentrations of free **1** are between 20 and 300 pg/mL.[34]

The application of the threshold assumption to EDCs may not be appropriate because the endocrine system, at baseline, has already achieved a physiological threshold. In some cases, adverse effects occur when hormone levels are still in the normal ranges defined for a population but are, in fact, elevated for a given individual. Thus, defining thresholds above which an effect could be expected becomes very difficult.[35]

Often, EDCs do not fit the traditional toxicological model of monotonic dose-response curves where the concept of a threshold, below which there is no effect, has meaning.[36] Low doses often produce effects of greater magnitude

Table 2. *In vitro* and *in vivo* assays for determining estrogenic and androgenic activity of chemicals.

Assay	**End point**	Comments ***- in vitro***
Binding assays (competitive, kinetic)	Measure of receptor-ligand binding affinity	Gives only measure of receptor binding affinity. No information on ligand behavior or transcriptional activation
MCF-7 human breast cancer cell	Cell proliferation	ERα specific. These cells grow and divide in a dose-dependent manner the presence of ER agonists.
Yeast Estrogen Screen (YES)	Transcriptional activation via increase in β-galactosidase activity	ERα specific. Activation of gene by ERα results in yeast producing the β-galactosidase enzyme which reacts with substrate that incorporates a chromophore which changes color.
Yeast Androgen Screen	Transcriptional activation via increase in β-galactosidase activity	Activation of gene by AR results in yeast producing the β-galactosidase enzyme which reacts with substrate that incorporates a chromophore which changes color.
Chinese Hamster Ovary Screen	Transcriptional activation via increase in firefly luciferase activity	Assay can be designed to be specific for ERα, ERβ or AR.
COS-1	Transcriptional activation via increase in chloramphenicol acetyltransferase activity	COS-1 cells transiently cotransfected with the wild-type receptor and an estrogen-responsive chloramphenicol acetyltransferase reporter gene.
Yeast 2-hybrid	Coactivator dependent transcriptional activation	Gives information on ability of a ligand to activate the receptor for binding with different coactivators to select specific genes for transcription.
Rainbow trout (or other fish, amphibians, reptiles, birds)	Vitellogenin induction	Vitellogenin is both the name of the gene responsible for production of the egg yolk protein precursor and the name of the protein produced. Both male and female fish have the gene but it is normally dormant in males. Male fish, on exposure to estrogen or estrogenic compounds produce the protein in a dose-dependent way. ERα biased because hepatocytes are used in the in vitro test.
		Comments - in vitro
Uterotrophic studies	Increase in mouse uterus weight on exposure to chemical	Biased due to ERα predominance of receptor in uterus

than do high doses. This results in an *inverted U-shaped* dose-response curve. This, in turn, leads to fundamental flaws in the traditional assumptions that high dose studies can be used to predict low dose effects, and that there is a threshold below which no adverse effect is observable.[37] Exposure to EDCs at concentrations when free hormones operate can have an entirely different suite of effects during active stages of development than they do when administered in high doses after an individual has fully developed.[36]

The definition of end point or adverse outcome in a risk assessment is critical in determining what is measured in characterizing the dose-response relationship of a chemical. For EDCs, the end point of interest may not be defined as clearly an adverse health outcome as for a suspected carcinogen. The end point of interest may be a perturbation in hormone levels, which could be a risk factor for a particular adverse health outcome. Since there cannot be a no-effect level when measuring a hormone response, the extrapolation of traditional studies to determine a no-effect level does not adequately protect future progeny from the adverse effects of EDCs.[37]

Summary

Although the sex hormone receptors are highly discriminatory towards endogenous hormones, they are quite promiscuous in the presence of 21st century chemicals. The potential for this sophisticated biological control mechanism to be hijacked so easily by foreign chemicals and so seriously impair our ability to reproduce, is alarming. Attributing adverse effects such as declining sperm count to these chemicals, and hence our ability to reduce these impacts is hampered by the conceptual and regulatory frameworks that have been adopted to deal with more *conventional* toxic chemicals.

It is now becoming clear that EDCs act along at least two different pathways. One is the direct effect on the exposed individual, which is amenable to standard risk assessment methodologies. However, the effects of EDCs occur at much lower doses and are more subtle than standard risk assessment frameworks consider. The second, and more insidious pathway, is the effect on the fetus resulting from maternal exposure during gestation. Current risk assessment methodologies do not consider the fetus as an individual and so it is disregarded in conventional assessments.

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37. Sheehan, D. M. *Proc. Soc. Exp. Biol. Med.* **2000**, *224*, 57-60.

NZIC Annual General Meeting

The NZIC AGM will take place at Victoria University in the Murphy Building, Lecture Theatre MY220, Kelburn Parade, at 5.30 for 6 pm start on Wednesday 11 November 2009. Entry to the Murphy Building is directly off Kelburn Parade (right hand side going up near the first pedestrian crossing) and the theatre is on Level 2.

AGENDA

1. Apologies
2. Minutes of 2008 AGM held at the University of Otago on Tuesday 2nd December 2008
3. Matters arising
4. Financial Report – including auditor's report
5. Election of Officers

    President

    1st Vice-President

    2nd Vice-President

    Treasurer

    Honorary General Secretary

6. Recommended change to Rule 20.8.6.

The rule currently reads:

20.8.6 To forward to the Council a copy of the Annual Report of the Branch, an audited copy of the Statement of Accounts and a list of papers read before the Branch during the year.

Council recommends that this be revised to:

20.8.6 Independent Financial Review

The financial statements of the Branch shall be reviewed and reported on annually by a person or firm, not being an officer or member of the Branch Committee, appointed by members of the Committee. The financial reviewer shall at all reasonable times have access to the books and accounts of the Branch and shall be entitled to such information and explanations as may be necessary for the performance of the review.

Then:

20.8.7 To forward to the Council a copy of the Annual Report of the Branch, a reviewed copy of the Statement of Accounts and a list of papers read before the Branch during the year.

And ***20.8.7*** becomes ***20.8.8***

7. Any other business

Nominations for the Officers of Council close with NZIC administration on 31 October 2009.