



# **ENDOCRINE DISRUPTORS AND THEIR EFFECTS ON FERTILITY**

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# BUT FIRST OF ALL...WHAT ARE ENDOCRINE DISRUPTORS?

- Endocrine disruptors are synthetic or natural exogenous chemicals, which are structurally and behaviourally similar to endogenous natural hormones. They are commonly known as endocrine disrupting chemicals (EDCs).
- The term 'endocrine disruption' became recognized worldwide following the 1991 Wingspread conference organized by Dr. Theo Colborn and colleagues.
- EDCs can either mimic natural hormones or interfere either with their interaction with their receptors or between the receptors and their target organs and cells, thereby transmitting confusing or incorrect signalling.
- We are mostly dealing with chemicals that mimic endogenous oestrogen, called environmental oestrogens or xenoestrogens, but some mimic or antagonise other hormones.
- Endocrine disruptors defy everything that is known about toxicology, since minute quantities appear to cause large effects out of all proportion to the dose.



# WHAT ARE THE PRINCIPAL ENDOCRINE DISRUPTING CHEMICALS?

- Pesticides, particularly organochlorines
- Toxic metals, particularly mercury, arsenic and cadmium
- Persistent organic pollutants (POPs)
- Volatile organic compounds (VOCs)
- Other: Tobacco smoke; fluoride; hazardous waste sites; incinerators; nitrates/nitrites; disinfectant byproducts; ionising radiation



# WHO - STATE OF THE SCIENCE OF ENDOCRINE DISRUPTING CHEMICALS 2012: KEY CONCERNS

- Many endocrine-related diseases and disorders are on the rise.
- Close to 800 chemicals are known or suspected to be capable of interfering with hormone receptors, hormone synthesis or hormone conversion. However, only a small fraction of these chemicals have been investigated in tests capable of identifying overt endocrine effects in intact organisms.
- *The vast majority of chemicals in current commercial use have not been tested at all.*
- The speed with which the increases in disease incidence have occurred in recent decades rules out genetic factors as the sole plausible explanation.
- Worldwide, there has been a failure to adequately address the underlying environmental causes of trends in endocrine diseases and disorders.
- A focus on linking one EDC to one disease severely underestimates the disease risk from mixtures of EDCs.
- It is likely that exposure to a single EDC may cause disease syndromes or multiple diseases, an area that has not been adequately studied.
- *But the WHO has no teeth!*



# WHY ARE EDCs OF PARTICULAR CONCERN (1)

- Damaging effects are found using very low chemical concentrations, which can be completely different from the effects of the same chemical at higher concentrations.
- Consequently, traditional approaches to determining safe exposure levels do not work with EDCs.
- Mechanisms of action are often unclear, making them difficult to counteract.
- Also, an EDC that affects one hormone is likely to affect another so some of its effect may be through the interaction of the two hormone systems.
- Unlike pesticides, which often display warning labels and include labelling for active ingredients, many other EDCs in consumer products enter homes unrecognized. The toxic ingredient may arise from product packaging materials rather than contents, and others may be present as by-products. (Dunagan SC, 2011)



# WHY ARE EDCs OF PARTICULAR CONCERN (2)

- There are many periods of vulnerability during which exposure to EDCs can be especially harmful, particularly prenatal and early postnatal development. Effects of early life exposure may be permanent but may not manifest until much later in life.
- EDC exposure in one generation may be transmitted to future generations (trans-generational effects) – animal studies have shown effect up to the F4 generation.
- The mechanism of transmission of transgenerational effects involves genomic and nongenomic modifications of the germ line, such as changes in DNA methylation and histone acetylation.
- Causal relationships are inherently difficult to establish in humans. A clear connection between the disorders and specific toxicants has not been established. This does not mean it does not exist but may be indirect rather than direct.



# ENDOCRINE SYSTEM COMPONENTS WHICH MAY BE AFFECTED BY EDCs

- Male and female reproductive function (especially fertility)
- Pancreas (insulin secretion, diabetes, obesity)
- Thyroid function
- Adrenal function
- Neuroendocrine function
- Male and female hormonal cancers
- Foetal development
- Effects in children

But the effects of EDCs may also extend beyond these systems to include multiple adult organs and tissues, such as kidneys and spleen (Anway, Skinner, 2006). What is unclear is whether they are acting as EDCs or as any toxic chemical.



# WHY DO WE NEED TO KNOW ABOUT ENDOCRINE DISRUPTORS?

We're not sleeping, we have gut problems, headaches, PMS, hot flushes, lethargy, unexplained weight gain, depression, brain fog, fatigue, PCOS, irregular periods, prostate trouble.

We're not sick – but we're not well either.

Welcome to the new normal!





# AND WE ARE IN THE MIDDLE OF AN EPIDEMIC OF EPIDEMICS!

- Epidemic of chronic disease
- Epidemic of obesity
- Epidemic of T2D
- Epidemic of AZ
- Epidemic of chronic fatigue
- Epidemic of depression
- Epidemic of hormonal cancers
- Epidemic of thyroid disease
- Epidemic of acronyms (ADD, ADHD, OCD, T2D, PMS, PCOS)

# PRINCIPAL ENDOCRINE DISRUPTOR SOURCES



# ROUTES OF EXPOSURE TO EDCs

Toxins can be taken into the body by:

- Ingestion (food and beverages)
- Inhalation
- Contact (skin/dermal): esp. lipophilic chemicals
- Injection: bypasses all the body's barriers

Exposure is affected by absorption. E.g metallic mercury swallowed from a broken thermometer is poorly absorbed in the gut and is largely excreted in faeces. But if the same amount of mercury were inhaled, a large quantity would be absorbed. And if the same quantity of mercury were injected, 100% would be absorbed.

Frequency and duration also influence the effect of exposure, as does time between exposures.



# PRINCIPAL TYPES OF PESTICIDE

Organochlorine insecticides	The oldest, and generally most toxic, pesticides. Developed as chemical warfare agents. Most are banned POPs. DDT, aldrin, dieldrin, chlordane, heptachlor, endosulfan, hexachlorobenzene, toxaphene
Organophosphate insecticides	Slightly less toxic than organochlorines: malathion, dursban, diazinon, trichlorofon, parathion, chlorpyrifos, malathion and mevinphos.
Carbamate insecticides	Similar to organophosphates. Includes aldicarb, carbaryl, methomyl, propoxur, thiophanate methyl and carbofuran (N-methyl carbamate).
Pyrethroid insecticides	The newest class, based on pyrethrin from chrysanthemums. Includes allethrin, cismethrin, fenvalerate and remethrin.
Herbicides	Some (e.g. paraquat) banned in the EU. Most common: Glyphosate, developed by Monsanto as RoundUp, designed for use with GM crops.
Fungicides	Widely used in agriculture/supermarkets: mancozeb, maneb, tributyltin.



# PESTICIDES

- Pesticide over-exposure, once the occupational hazard of agricultural workers, is now everyone's problem.
- Although several pesticides are now banned in the US and EU, this does not stop firms manufacturing banned pesticides for export to other countries. The produce from these countries may then be imported back into the EU, having been sprayed or grown with banned chemicals.
- Despite European policies to reduce pesticide use, it has not in fact decreased, with new toxic chemicals replacing those now banned.
- And since the advent of pesticides, the total number of pests has not significantly lessened, but they have now become highly resistant to pesticides. This prompts the use of more pesticides.
- Governments continue to insist that pesticide levels are falling and are now almost undetectable. But these surveys are normally carried out at the instigation of conventional farmers who complain that the organic food industry is stealing business from them.



## Quotation from the European Crop Protection Association (the pesticide producers' union)



**‘All crops need protection  
from disease and pests’.**



# ORGANOCHLORINE PESTICIDES

Most organochlorines have been banned in the US and EU but may still be imported illegally.

- DDT was first synthesised in the 1940s and developed as a chemical warfare agent.
- DDT and its metabolites DDE and DDD bioaccumulate in ecosystems for decades and damage non-target species '*...there is now not a single living organism on the planet that does not contain DDT*' (Turusov V, Environ Health Perspect, 2002). DDT has been banned in the EU since the 1970s.
- DDT remains an important class of insecticide for the eradication of malaria in both developed and developing countries; the US still manufactures DDT for export.
- UK surveys found that in 2009, 46% of foods contained pesticide residues, many among the most highly toxic. This percentage rises every year and has almost doubled since 2003 when it was 25%.
  - DDT (OC), was found in burgers, oily fish, smoked fish (particularly salmon) and liver.
  - 2,4-D (OC herbicide), also banned, was in over 50% of the organ meats tested,
  - Lindane (OC) was found in in 75% of chocolate samples
  - More worryingly, pesticides were found in over 12% of jars of baby food.
- In the US a study found DDT in over 70% of 4-year olds, principally from breast milk (Jacobson, Am J Public Health, 1989).

## *New York Post June 2016*



The answer to Zika is obvious:  
bring back DDT.

The Zika virus outbreak makes it  
clearer than ever: It's time to end  
the ban on DDT — a ban that was  
never sensible in the first place,  
but now is downright  
unjustifiable’.





# ORGANOPHOSPHATE PESTICIDES



- Organophosphates (OPs) are also derived from chemical warfare nerve agents and were used in the Gulf War, possibly contributing to Gulf War Syndrome.
- Sarin gas was used in the Tokyo subway attacks in 1995.
- Sarin and other OPs have been stockpiled by the military of various countries and continue to present problems of disposal.



# HERBICIDES



- Also developed for warfare, they were the principal components of Agent Orange, used in the Vietnam war.
- Best known is glyphosate, developed by Monsanto as RoundUp.
- Glyphosate has been found contaminating streams in both agricultural and urban areas.
- In a 2016 survey, the US FDA found glyphosate in 100% of all samples of honey, some with double the amount legally allowed in the EU.
- Herbicides are now an essential part of the agriculture industry but a serious limitation is their lack of specificity i.e. they also damage the crop they are intended to protect.
- Because of its extreme toxicity, there have been petitions to ban glyphosate.



# 'INERT' PESTICIDE INGREDIENTS

- Few pesticide chemicals are applied alone; instead they are applied in formulations using different combinations of several pesticide ingredients, both active and 'inert'.
- The inert ingredients are toxic in themselves and can enhance the toxic potential of the active ingredients. But they are not listed on the label as they are 'trade secrets'. In many cases these inert ingredients make up >90% of the pesticide product.
- Many inert ingredients are extremely toxic and include known human carcinogens.
- The health effects of pesticides may be as much to do with these 'inert' ingredients as those declared on the label.



# TOXIC METALS: PRINCIPAL SOURCES

Non-organic foods and cans/linings	Aluminium, arsenic, cadmium, chromium, copper, iron, lead, mercury, nickel, palladium, silver, titanium
Infant formula/food	Aluminium, arsenic, cadmium, lead, manganese, mercury
Cookware	Aluminium
Drinking water	Aluminium, arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, palladium, uranium
Pharmaceuticals	Aluminium, copper, mercury, palladium, titanium
Cosmetics and toothpaste	Aluminium, arsenic, cadmium, chromium, lead, mercury, nickel, silver, titanium
Toiletries	Aluminium, arsenic, nickel
Vaccines	Aluminium as adjuvant, mercury (as thimerosal)
Dental materials	Cadmium, chromium, copper, mercury, nickel, palladium
External or internal air	Aluminium, arsenic, cadmium, iron, lead, mercury, nickel, silver, titanium, uranium



# VACCINES: MERCURY

- Thimerosal, a preservative (antimicrobial) is 49.6% mercury.
- In the UK, since 2004 only hepatitis B and influenza jabs are allowed to contain thimerosal, which must be phased out.
- BUT...Green Health Watch found that in the US, manufacturers still made vaccines with thimerosal to reduce costs but filtered it out afterwards. So they are technically thimerosal-free. BUT.....
- This did not necessarily remove all the mercury, which continued to be present in vaccines, albeit in lower quantity.
- Also, US vaccines exported to less developed countries could still contain thimerosal. Mercury's antimicrobial function was deemed more important than its neurotoxicity. The WHO raised no objection.



## VACCINES: ....AND ALUMINIUM TOO

- Aluminium is added to vaccines as an adjuvant: an agent that stimulates the immune system to increase its response to the vaccine. This reduces costs for the manufacturer.
- Aluminium in vaccines has never been tested for safety.
- Studies on the HPV vaccine found that the aluminium adjuvant had no beneficial effect on immune response.
- There is risk of 'accidental exposure': a study found that some vaccines contained 5-6 times more aluminium than shown on the label.
- In contrast to ingested aluminium, the body absorbs 100% of injected aluminium.



# DENTISTRY

- Dental amalgams contain mercury.
- The amalgam releases mercury vapour continuously, which is exacerbated by eating, drinking and brushing and grinding teeth.
- Several European countries (not the UK!) have required amalgam fillings to be phased out or banned. In Sweden they were banned in 1994.
- Sweden also requires all amalgam fillings to be removed from corpses before cremation to prevent environmental contamination.
- The UN Minamata Convention requires the phasing out of many mercury sources by 2020. But the treaty takes effect only after its ratification by 50 nations, which has not yet occurred.
- The EU Commission is currently debating ratification of legislation requiring 'phasing down' of mercury use, rather than 'phasing out'.



# PERSISTENT ORGANIC POLLUTANTS (POPs)

- POPs are carbon-based (i.e. organic) chemicals which are resistant to environmental degradation, taking up to 100 years to biodegrade (Fisher BE. Environ Health Perspect. 1999).
- They are lipophilic and bioaccumulate in the environment and in fatty tissue.
- POPs comprise some pesticides, industrial chemicals, metals and pharmaceuticals.
- Many POPs are also volatile organic compounds (VOCs).
- The UN Binding Convention on Persistent Organic Pollutants banned 11 POPs in 2001 because of their acknowledged damage to the environment. Since 2001, this list has been expanded but many others remain in use.
- Because they are 'persistent', even those banned in 2001 remain in the environment and our bodies.

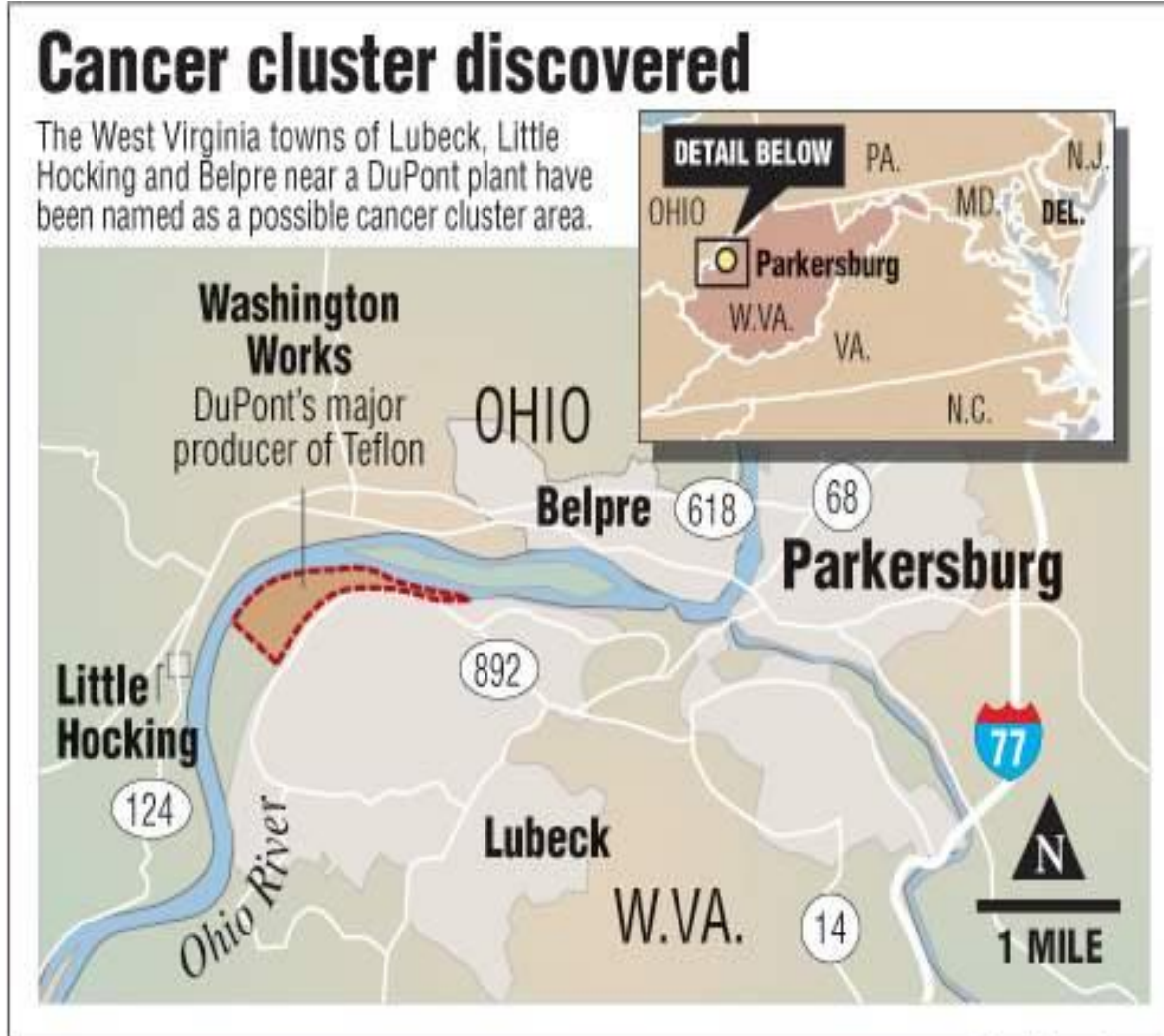




# THE PRINCIPAL POPs

Polychlorinated biphenyls (PCBs)	Used in insulation; found in old electrical equipment, office products and pesticides. Found in meat, fish, dairy, cord blood and breast milk.
Polychlorinated dibenzofurans (PCDFs, furans)	Produced by incineration of organochlorine chemicals: paints, solvents, pesticides, plastics. Found in electrical equipment.
Polychlorinated dibenzo- <i>p</i> -dioxins (PCDDs, dioxins)	Produced by incineration of organochlorine chemicals: paints, solvents, pesticides, plastics. Used in manufacture of pesticides and white paper products. Found in animal fat.
Phthalates	Used in any plastics, including cling film and other food packaging and manufacture of cosmetics, pharmaceuticals, toiletries and pesticides. Found in air, food and water.

# Perfluorooctanoic acid (PFOA)



The News Journal

- Dupont, makers of non-stick Teflon (as well as Stainmaster and Scotchgard), faced its third lawsuit in July for health damage resulting from dumping product waste, specifically PFOA, in a river, polluting the drinking water and allegedly causing testicular cancer. The jury decided that Dupont 'acted with malice' and awarded over \$5.5 million against them.
- Around 3,500 lawsuits against Dupont are still pending.



# VOLATILE ORGANIC COMPOUNDS (VOCs)

- VOCs are organic (i.e. carbon-containing) chemicals that have a high vapour pressure at room temperature.
- This means they can easily evaporate as gases into the air from some solids or liquids and may travel thousands of miles before depositing on the earth or water
- Many of them are solvents, which may be smelled to induce intoxication e.g. glue sniffing.
- Because they are ingredients in many commonly used products, they are present in virtually all indoor air, particularly from cleaning products, toiletries and perfume.
- Levels of VOCs increase as temperature rises, so if a building is deemed 'safe' on a cold day, it says nothing about its condition on a hot day.



# PRINCIPAL SOURCES OF VOCs

**Formaldehyde**

**Embalming, paints, adhesives, plastics, soft furnishings, building materials, insulation, natural gas, kerosene, cigarette smoke**

**Vinyl, polyvinyl chloride (PVC)**

**Wallpaper, upholstery, carpets, building materials, vehicles, plastic products, rubber, paper, glass.**

**Polycyclic aromatic hydrocarbons (PAHs)**

**All forms of cooking, incense, cigarette smoking, vehicle exhausts, industrial emissions, mothballs, tar, pitch, creosote, pesticides, toiletries, synthetic turf. Incineration of plastic waste such as PVC.**

**Ethanol (alcohol)**

**Perfume, air fresheners, toiletries, hairspray, nail polish/removers, detergents, paints, varnishes/removers, industrial cleaners, de-icers.**

**Benzene**

**Cigarette smoke, fuels, vehicle exhausts, plastics, adhesives, cleaners.**

**Perchloroethylene**

**Also tri- or tetrachloroethylene. Dry cleaning, carpets, paints, degreasing.**



# TOBACCO SMOKE AND OTHER EDCs IN THE HOME

Tobacco smoke components and other EDCs represent a particular problem in the home, because:

- Most people spend a significant proportion of their time indoors, particularly those that work from home and the most vulnerable: the very young, the elderly, the infirm.
- Energy conservation measures have dramatically decreased ventilation; we are now double glazed, weather-proofed and draught-excluded.
- So now during half the year no toxins can escape the home, leading to a build-up in air and dust, which settles on floors and soft furnishings, particularly carpets.
- Toddlers and infants are most at risk from flooring and carpets, as they spend much of their time on the floor and tend to put objects and fingers into their mouths.



# DISINFECTANT BY-PRODUCTS (DBPs)

- In Europe there is a legal requirement for public water to contain disinfectant chemicals to destroy micro-organisms. This has largely resulted in the eradication of numerous waterborne diseases including cholera and typhoid but.....
- Chlorine is the most common disinfectant chemical, but all carry their own health risks as they produce by-products as a result of the interaction of the disinfectant with organic matter in the water (sewage, animal waste, soil, plant material), producing halomethanes, particularly chloroform.
- Showering is an important route of exposure to chloroform and other DBPs. The quantity inhaled during a 15-minute shower is equivalent to drinking 8 glasses of contaminated water (Andelman JB, Environ Health Perspect 1985).
- While bathing and showering, these chemicals can also be absorbed through the skin and into the circulation; skin absorption can contribute up to 91% of the total exposure to VOCs (Brown HS, Am J Public Health, 1984).
- Only trihalomethane levels are regularly monitored in the UK.



# FLUORIDE IN DRINKING WATER

- Fluoride was originally developed during WW2 for weapons research and was subsequently used for the manufacture of weapons-grade uranium and plutonium during the Cold War.
- Fluoride is also used as a rat poison.
- To prevent tooth decay, around 10% of the UK drinking water supply is deliberately contaminated with hydrofluorosilicic acid (an industrial waste product from phosphate fertiliser manufacture, containing many toxic metals).
- Most of the remainder of western Europe has rejected fluoridation on the grounds that:
  - it does not work and
  - it is a violation of human rights to forcibly medicate whole populations of people.
- Joint statement by US CDC and American Dental Association:  
*'Fluoridation is nature's way to prevent tooth decay'.*



# FLUORIDE IN DRINKING WATER AND TOOTHPASTE

- Some of the research shows that fluoride benefits teeth but these studies are based on naturally occurring calcium fluoride, whereas what is added to the water supply is hydrofluorosilicic acid.
- Some studies are now showing that among children there is little difference in incidence of dental caries between children living in fluoridated and non-fluoridated areas.
- And dental decay does not increase when communities stop fluoridation (Burt BA, J Dent Res 2000; Kunzel W, Commun Dent Oral Epidemiol, 2000; Seppa L, Caries Res 2000).
- The maximum allowable level of fluoride in water is 4 parts per million. But the fluoride concentration in toothpaste is considerably higher.
- The fluoride in toothpaste is generally sodium fluoride, some of which may be ingested, particularly by children. Only 5g may be a lethal dose (Bentley EM, Br Dent J, 1999).
- Several hundred children each year are treated at healthcare facilities for suspected over-ingestion of fluoride toothpaste (Shulman JD, Wells LM, J Public Health Dent, 1997).
- In the US, fluoride-containing toothpastes now come with a health warning and information on how to contact your local poisons control office in the event of accidental ingestion.





# AND FINALLY....

Just because there are headlines saying a toxin is being or has been banned, don't assume you can discount it as a source:

- It can take many years from a headline to actual banning (e.g. Minamata Convention)
- The ban may be disregarded as it is cheaper to pay the fine
- Many toxins have a long half life in the body, so will be present for many years.
- The toxin may have been acquired in a country with no ban.
- The toxin may have been acquired from an import (e.g. lead in traditional Chinese medicines)
- Low dose exposure may take many years to manifest as symptoms.



# **PRINCIPAL ENDOCRINE DISRUPTOR EFFECTS ON FERTILITY**



# CAVEAT

For every study showing an association with a particular EDC, there is probably another study showing no association.

I am only showing you the studies where there is an association.



# FEMALE REPRODUCTIVE FUNCTION

- Female reproductive function depends upon the exquisite control of ovarian steroid production, which enables follicle production, ovulation and pregnancy.
- Ovarian development and function are collectively regulated by a host of endogenous growth factors, cytokines, gonadotropins and steroid hormones as well as exogenous factors such as nutrients and environmental agents.
- EDCs can disrupt ovarian processes, leading to anovulation, infertility, oestrogen deficiency and premature ovarian failure, principally by altering the availability of ovarian hormones or by altering the binding and activity of the hormone at the receptor level (Craig ZR, Reproduction, 2011).
- The most sensitive exposure timing is *in utero* or as a young child, even though the effects may not manifest for many years.
- The impaired fertility rate in the U.S. increased from 11 to 15% between 1982 and 2002 (Guzick and Swan, 2006). Although various other confounding factors such as lifestyle changes can contribute to this decline, the role of EDCs is also strongly considered.



# AN EXTREME EXAMPLE: DIETHYLSTILBESTROL (DES)

- **DES is a synthetic nonsteroidal oestrogen used as a medical treatment for many conditions. Between 1940-1971 it was given to pregnant women to prevent miscarriage and other pregnancy complications and losses.**
- **DES was banned after a relationship was discovered between taking DES and development of adenocarcinoma of the vagina and cervix in young women whose mothers had taken DES while pregnant.**
- **It was later found that women exposed in utero also had an increased risk of breast cancer at age  $\geq 40$ , reproductive tract abnormalities and increased vaginal and cervical cancer risk.**
- **Daughters of exposed women reached puberty later, had more irregular periods and fewer live births.**
- **Males exposed *in utero* had an increased incidence of genital abnormalities and a possibly increased risk of prostate and testicular cancer.**
- **Increased susceptibility for tumours is passed on from the maternal lineage to subsequent generations of male and female descendants (transgenerational effect).**
- **An animal study showed that prenatal exposure to DES may permanently alter gene expression and methylation.**

(Schrager S, Am Fam Physician, 2004; Newbold R, Environ Health Perspect, 1995; Newbold RR, Endocrinology, 2006; Titus-Ernstoff L, Int J Epidemiol, 2006; Palmer JR, Cancer Epidemiol Biomarkers Prev, 2006; Li S, Ann NY Acad Sci, 2003; Li S, Mol Carcinog, 2003)



# INTERESTING POINTS IN THE FOLLOWING STUDY RESULTS

- Some EDCs cause an increase in the parameter being assessed, while some cause a decrease. Sometimes this can happen with different metabolites of the same EDC.
- Not all associations are linear. EDCs may show a non-linear but monotonic effect, a non-linear, non-monotonic effect or a threshold effect. (*More of this later*)
- This often manifests as a positive association at high dose but an inverse association at low dose.
- Where toxins are studied in combination, there may be an agonistic or antagonistic effect. If agonistic, they may be additive or synergistic.
- A note on terminology: All associations are statistically significant. The assumption is that all associations are positive; where it is not, I specify that the association is inverse.



# FEMALE REPRODUCTIVE FUNCTION

- Earlier age of puberty and breast development: Exposure to PCBs, polybrominated biphenyls (PBBs), DDT and phthalate esters.
- Natural menopause at an earlier age: Higher blood dioxins and OC pesticides
- Abnormal menstrual cycles: PCB exposure was associated with **shorter** cycles and abnormal menstrual bleeding, DDE was associated with **longer** cycles. Solvents containing 2-bromopropane were associated with absence of periods and hot flushes.
- Reduced oestrogen and progesterone levels (critical for ovulation and early pregnancy maintenance): association with DDT.

(Den Hond E, 2006; Krstevska-Konstantinova M, 2001; Rasier G, 2008; Colon I, 2000; Cooper GS, Epidemiology, 2002; Woodruff TJ, 2008; Toft G, Hum Reprod, 2008; Yu ML, Int J Epidemiol, 2000; Kim Y, Scand J Work Environ Health, 1996; Perry MJ, Am J Epidemiol, 2006)

# And on the subject of phthalates...



**Rubber ducks can kill your sex drive, research finds (Guardian, 2014)**

Women with the highest levels of phthalates - chemicals used to make plastics bendy – were far more likely to suffer low libido, study reveals.

(Barrett E, Horm Behav, 2014)





# EFFECT OF SMOKING ON FEMALE REPRODUCTIVE FUNCTION

- A systematic review showed that cigarette smoking (particularly the toxins cadmium, nicotine, cotinine) dose-dependently affected each stage of reproductive function: folliculogenesis, steroidogenesis, embryo transport, endometrial receptivity, endometrial angiogenesis, uterine blood flow and uterine myometrium (Caserta D, Hum Reprod Update, 2011).
- These are associated with lower fertility rates, adverse reproductive outcomes and a higher risk of IVF failures. (Dechanet C, Hum Reprod Update, 2011).
- Note that cigarette smoking, source of several thousand chemicals, is a perfect example of why we shouldn't look at 1 chemical in isolation!



# GYNAECOLOGICAL CONDITIONS HINDERING PREGNANCY

## Polycystic ovary syndrome (PCOS):

- BPA levels in women with PCOS were significantly higher compared to healthy women and there was a positive correlation between BPA serum levels and androgen concentrations (Takeuchi T, Endocr J, 2004; Caserta D, Reprod Biol Endocrinol, 2014)
- Women with PCOS had **higher** serum levels of **perfluorinated compounds (PFCs)** but **lower** urinary concentrations of **phthalates** than controls (Vagi SJ, BMC Endocr Disord, 2014).

## Endometriosis and uterine fibroids:

- Significantly higher serum and plasma levels of **phthalates** were found in women with endometriosis compared to healthy controls (Reddy BS, BJOG, 2006; Cobellis L, Hum Reprod, 2003).
- Urinary **phthalate** metabolite MBP was higher in women with either or both conditions but urinary MEHP was lower than controls (Weuve J, Environ Health Perspect, 2010).
- Following an industrial explosion in Seveso, Italy, serum **TCDD** was associated with fibroids 20 years later, suggesting an anti-oestrogenic effect in the uterus, although there were oestrogenic effects elsewhere in the body (Eskenazi B, Am J Epidemiol, 2007).



# FEMALE FERTILITY

## BPA – the prime suspect:

- BPA was originally developed as a synthetic oestrogen; its oestrogenic effect was used to induce rapid growth in cattle and poultry. It was also used for a few years as oestrogen replacement for women (Singh S, 2012). It appears to be the key chemical involved in infertility.
- Urinary BPA inversely correlated with the number and quality of eggs retrieved before IVF and was found to impair implantation (Varayoud J, Vitam Horm, 2014; Mok-Lin E, Int J Androl, 2010).
- Blood levels of BPA were higher in infertile vs fertile women (La Rocca C, Int J Environ Res Public Health, 2014; Caserta D, Gynecol Endocrinol, 2013).



# TIME TO PREGNANCY (TTP)

- Time-to-pregnancy (TTP) is defined as the number of prospectively observed menstrual cycles required for pregnancy.
- A 2012 systematic review found that serum **lead** levels, followed by **pesticide** exposure showed a strong association with TTP (Snijder CA, Hum Reprod Update, 2012).
- The sum of all blood **polybrominated diphenyl ether (PBDE)** flame retardants was associated with TTP (Harley KG, Environ Health Perspect, 2010).
- In a US agricultural region, infertile women were more likely to have mixed and applied **herbicides or fungicides** 2 years before attempting conception (but residing on a farm or a ranch was protective) (Greenlee AR, Epidemiology, 2003).
- TTP was associated with estimated **PCB** exposure from sport fish consumption (Buck GM, Epidemiology, 2000).



# IN VITRO FERTILISATION

- **BPA:** Higher urinary or serum BPA was associated with decreased ovarian response, number of fertilized eggs and decreased blastocyst formation, increased implantation failure and reduced oestrogen receptor E(2) response (Bloom MS, Fertil Steril, 2011; (Ehrlich S, Hum Reprod, 2012; Fujimoto VY, Fertil Steril, 2011; Mok-Lin E, Int J Androl, 2010; Ehrlich S, Environ Health Perspect, 2012).
- **Phthalates:** Urinary phthalate levels were lower in women who conceived after treatment (Alur S, Fertil Steril, 2015).
- **Pesticides:** Serum hexachlorobenzene (HCB) levels were associated with increased implantation failure (Mahalingaiah S, Environ Health Perspect, 2012).
- **PCBs:** Serum PCB concentrations were associated with increased implantation failure (Meeker JD, Environ Health Perspect, 2011).
- **Lead:** Higher blood lead levels were associated with a 75% reduction in viable eggs (Bloom MS, Reprod Toxicol, 2010).
- Fertilization was **inversely** correlated with serum and follicular fluid **DDE**, but pregnancy was **positively** correlated with follicular fluid **PCBs** (Younglai EV, 2002).

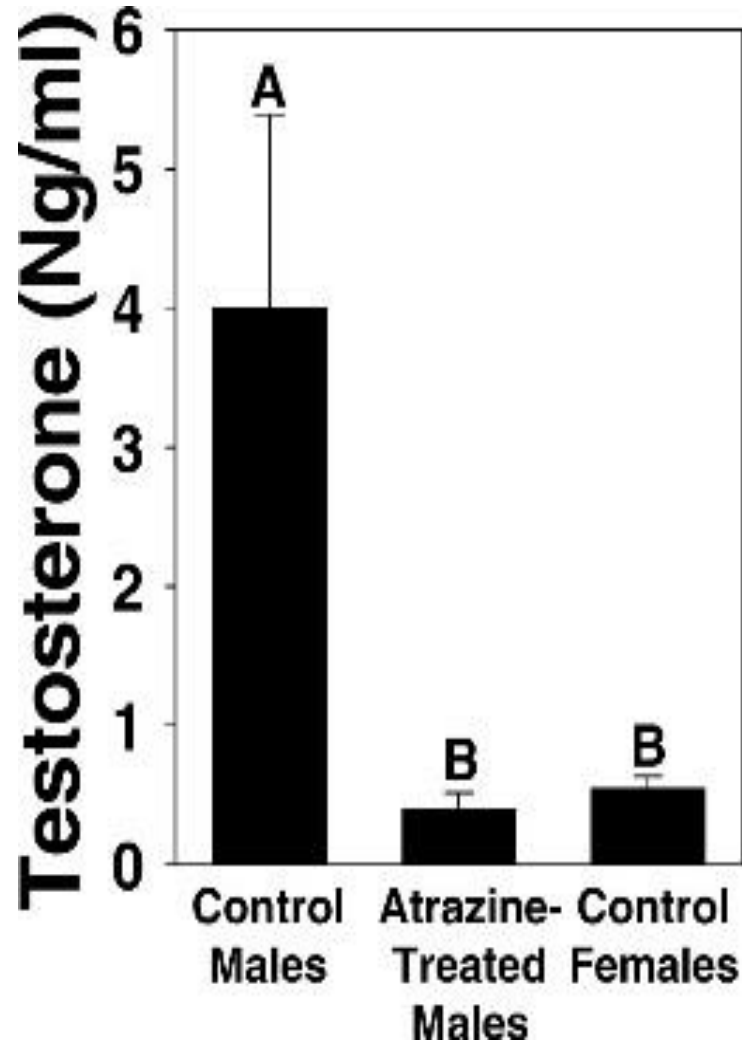


# MALE REPRODUCTIVE HEALTH (1)

- Between 30 and 50% of infertile couples are infertile due to the male, mainly due to sperm production disorders (Multigner L, Scientific World Journal, 2002).
- Semen quality is declining throughout the world, putting male fertility at risk. Animal studies suggest that EDCs may be the culprit but causal relationships are inherently difficult to establish in humans and many show inconsistent results. (Nordkap L, Mol Cell Endocrinol, 2012; Giwercman A, Reprod Biomed Online, 2007)
- 50 years ago the principal exposures for males were occupational. Industrial exposures in the developed world are now much reduced but now the chemicals concerned have become ubiquitous in our daily lives.
- Male fertility is also highly sensitive to radiant heat, ionising radiation and high frequency non-ionising radiation (Bonde JP, Asian J Androl, 2010).
- These exposures have largely oestrogenic and anti-androgenic effects on males, resulting in reduced fertility and more feminine characteristics among males.



## Examples of feminised male frogs



Effect of 46-day exposure to low dose atrazine on plasma testosterone levels in sexually mature male frogs. The dose was equivalent to normal human exposure.

(Hayes TB, Proc Natl Acad Sci USA, 2002)

*Note that atrazine-treated males have less testosterone than females!*

# And it certainly fooled some!



**A healthy male frog attempting (but failing!) to mate with a male frog exposed to atrazine.**

**The exposed frog had become fully feminised, tricking the healthy male into thinking it was a female.**





# MALE REPRODUCTIVE HEALTH (2)

- **PCBs:** consistent inverse associations with total testosterone, SHBG-bound testosterone and the testosterone/SHBG ratio.
- **Pesticides:** **occupational exposure studies** showed a positive association with oestradiol and testosterone and an inverse association with FSH. But **non-occupational studies** showed an inverse association with oestradiol, testosterone, inhibin B and the free androgen index and a positive association with SHBG, FSH and LH. (*Inhibin B downregulates FSH synthesis and inhibits FSH secretion*)
- **Phthalates:** inversely associated with testosterone, oestradiol, FSH and LH; one study showed a positive association with SHBG and another an inverse association
- **Dioxins:** occupational exposure: positive association with FSH and LH and inverse association with testosterone

(Turyk ME, Environ Res, 2006; Persky V, Environ Health Perspect, 2001; Goncharov A, 2009; Bonde JP, 2008; Richthoff J, 2003; Oliva A, Hum Reprod, 2001; Garry VF, Toxicol Ind Health, 1999; Meeker JD, Epidemiology, 2006; Meeker JD, Reprod Toxicol, 2008; Specht IO, Reprod Toxicol, 2015; Meeker JD, Reprod Toxicol, 2009; Han Y, Chemosphere, 2008; Giwercman AH, Environ Health Perspect, 2006; Meeker JD, J Androl, 2009; Jonsson BA, 2005; Specht IO, Environ Int, 2014; Duty SM, Hum Reprod, 2005; Egeland GM, Am J Epidemiol, 1994).



# MALE REPRODUCTIVE HEALTH (3)

- **Metals:** **Inverse** association between molybdenum and serum testosterone, particularly among men with low zinc. **Positive** association between cadmium, copper and lead and serum testosterone. **Positive** association of mercury and SHBG and inhibin B.
- **BPA:** **Occupational exposure:** an inverse association between BPA and FSH; **non-occupational exposure:** positive association with FSH and testosterone and an inverse association with inhibin B and the free androgen index (FAI) (FAI = testosterone/SHBG ratio).
- **PBDEs:** Inverse association with LH, FSH and FAI and positive association with testosterone, inhibin B and SHBG.

(Meeker JD, Fertil Steril, 2010; Lewis RC, Fertil Steril, 2015; Telisman S, Environ Health Perspect, 2000; Mocevic E, Asian J Androl, 2013; McGregor AJ, Hum Exp Toxicol, 1991; Hanaoka T, Occup Environ Med, 2002; Meeker JD, Environ Sci Technol, 2010; Mendiola J, Environ Health Perspect, 2010; Takeuchi T, Biochem Biophys Res Commun, 2002;(Meeker, JD, 2001; Turyk ME, Environ Health Perspect, 2008)



# SPERM CONCENTRATION AND QUALITY (1)

- ‘Semen quality may be the most sensitive marker of environmental exposures’ (Nordkap L, Mol Cell Endocrinol, 2012).
- In developed countries, male infertility is normally due to low sperm motility and/or sperm count (Tavares RS, Reprod Toxicol, 2009).
- There is a high incidence of low sperm counts in European men over recent decades. ‘Spermatogenesis in normal men is poorly organized and inefficient so that men are poorly placed to cope with environmental insults’. (Sharpe RM, Philos Trans R Soc Lond B Biol Sci, 2010).
- Timing of exposure is important: after the chemical plant explosion in Seveso, Italy, in which the local population were exposed to TCDD, males exposed prior to puberty had poor semen quality as adults but those exposed aged  $\geq 10$  had normal sperm quality (Mocarelli P, 2008).

# SPERM CONCENTRATION AND QUALITY (2)

- **Phthalates:** In an infertility clinic, urinary phthalate concentrations were associated with low sperm concentration and total sperm count.
- Phthalates were inversely associated with sperm volume, quality, concentration, morphology, motility and sperm count. The combination of higher PCB and phthalate metabolites had a **greater than additive effect** on decreased sperm motility. Urinary phthalates were inversely associated with sperm motility.
- **Metals:** molybdenum was inversely associated with sperm concentration and morphology, particularly in males with low copper. Median sperm concentration was reduced by 49% in European men with blood lead concentrations  $>50\mu\text{g}/\text{dl}$ ; this is an example of a threshold dose response rather than a linear relationship. Lead and cadmium concentrations were inversely associated with sperm density and motility.
- **PCBs:** inversely associated with sperm motility and morphology; the association with sperm count was particularly seen in men with short androgen receptor CAG repeat length.

(Wang YX, Environ Res, 2015; Hauser R, Environ Health Perspect, 2005; Hauser R, Epidemiology, 2006; Thurston SW, Andrology, 2015; Specht IO, Environ Int, 2014; Wirth JJ, Syst Biol Reprod Med, 2008; Giwercman A, Pharmacogenetics Genomics, 2007; Richthoff J, Environ Health Perspect, 2003; Hauser R, Environ Health Perspect, 2003; Lenters V, Occup Environ Med, 2015; Meeker JD, Environ Health Perspect, 2008; Telisman S, Environ Health Perspect, 2000; Bonde JP, Occup Environ Med, 2002)

# As well as GM foods and Round-Up, we can also blame Monsanto for PCBs

**BREAKING NEWS**

## **MONSANTO LOSES PCB LAWSUIT**

**Ordered to pay \$46.5 million by St. Louis jury in a suit alleging negligence in the production of PCBs. Three plaintiffs claimed PCBs caused their cancer.**



**“... justice is going to be served whether it’s a year after the products are put out, or in this case, 80 years.” - Juror Ashley Enochs**

[www.gmofreeusa.org](http://www.gmofreeusa.org)

[www.facebook.com/gmofreeusa](http://www.facebook.com/gmofreeusa) [www.tsu.co/gmofreeusa](http://www.tsu.co/gmofreeusa) [www.facebook.com/gmofreecanadagroup](http://www.facebook.com/gmofreecanadagroup)

# SPERM CONCENTRATION AND QUALITY (3)

- **Perfluorinated compounds:** PFOS and PFHxS levels were **inversely** associated with percentage normal cells, although PFOA levels were **positively** associated with percentage normal cells.
- **Pesticides:** inversely associated with sperm density, morphology and motility, sperm count and percentage of normal sperm.
- **Organophosphate flame retardants:** inverse association with sperm concentration; **polybrominated flame retardants:** inversely associated with semen quality and testis size.
- **BPA:** inversely associated with sperm concentration, motility and morphology.

(Toft G, Hum Reprod, 2012; Chiu YH, Hum Reprod, 2015; Meeker JD, Hum Reprod, 2008; Aneck-Hahn, NH, J Androl, 2007; Meeker JD, Environ Health Perspect, 2004; Meeker JD, Environ Health Perspect, 2010; Akutsu K, 2008; Meeker JD, Reprod Toxicol, 2010)



# SPERM DNA DAMAGE

- **Pesticides:** associations with DDT (OC), chlorpyrifos (OP), carbamates and pyrethroids. The DNA fragmentation index (DFI) was 40% higher with higher levels of serum DDE in a subgroup of men with short androgen receptor CAG repeat length. Chromosome aberration frequency ('**genomic instability**') was associated with volume of herbicides applied but reversed after exposure ceased.
- **PCBs:** positive association with DNA damage, apoptotic markers. Among Swedish men, PCBs were **positively** associated with higher Y-chromosome-bearing spermatozoa (i.e. higher proportion of males) but in Polish males there was an **inverse** association.
- **Parabens and BPA:** positive association with sperm DNA damage.
- **Perfluorinated compounds:** Associations with **both** DNA hypo- and hyper-methylation. A positive **non-linear** association between PFOS and the sex chromosome ratio. Positive association of PFOA with sperm DNA fragmentation.
- **Phthalates:** positive association with DNA and fragmentation.

(Garry VF, Environ Health Perspect, 2001; Bonde JP, Environ Health Perspect, 2008; Meeker JD, Hum Reprod, 2008; Meeker JD, Hum Reprod, 2004; Giwercman A, Pharmacogenetics Genomics, 2007; Tijdo T, Environ Health Perspect, 2006; Bonde JP, Environ Health Perspect, 2008; Stronati A, Reproduction, 2006; Rignell-Hydborn A, Environ Health Perspect, 2005; Meeker JD, Environ Health Perspect, 2011; Meeker JD, Reprod Toxicol, 2010; Specht IO, Reprod Toxicol, 2012; (Leter G, Environ Mol Mutagen, 2014; Kvist L, Reprod Toxicol, 2010; Hauser R, Hum Reprod, 2007; Pant N, Toxicol Appl Pharmacol, 2008; Duty SM, Environ Health Perspect, 2003)



# MALE FERTILITY

It has been estimated that 7-15% of U.S. males are infertile. There are associations with:

- **Phthalates**: higher levels in infertile men.
- **Pesticides**: fertility associated with **higher levels** of certain herbicides and insecticides but **lower levels** of other insecticides. Fertilisation rates were significantly decreased for couples with paternal pesticide exposure.
- **BPA**: serum concentrations were inversely associated with embryo cell number in an IVF clinic.
- Male serum/urine **lead, phenol, PCB and phthalate levels** were associated with increased time to pregnancy.

(Cantonwine DE, Expert Rev Obstet Gynecol, 2013; Pant N, Toxicol Appl Pharmacol, 2008; Swan SH, Environ Health Perspect, 2003; Tielemans E, Lancet, 1999; Bloom MS, Environ Toxicol Pharmacol, 2011; Buck Louis GM, Andrology, 2016)



# TAKE HOME MESSAGES FROM EDCs AND FERTILITY

- Fertility is affected by, principally, BPA but also by maternal smoking, phthalates, PCBs, PFCs, dioxins, metals, PBDEs and pesticides in both males and females.
- Some EDCs cause an increase in the parameter being assessed, while some cause a decrease. Sometimes this can happen with different metabolites of the same EDC. What we should understand from this is that any variation from the normal range, in whichever direction, is an indication of impaired health.
- The association may manifest as a positive association at high dose but an inverse association at low dose or a purely low dose effect. So we can't assume a linear association.
- Where toxins are studied in combination, there may be an agonistic or antagonistic effect. Not enough is yet known about combinations of toxins, yet this surely represents the real world.



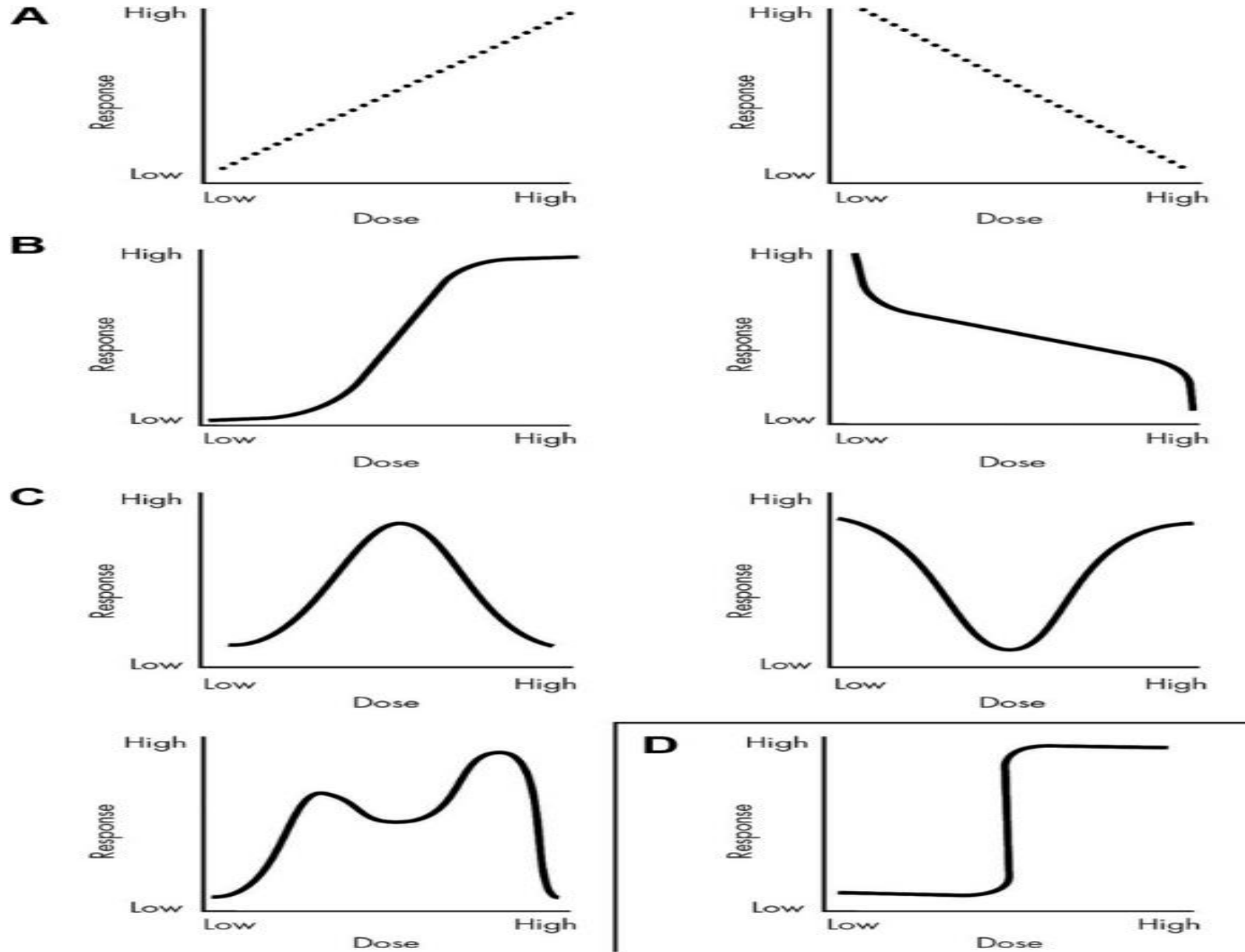
# ENDOCRINE DISRUPTORS: LOW DOSE EXPOSURE

- A 2007 Expert Review stated: 'Based on the evidence, it is concluded that these "low dose" effects have yet to be established, that the studies purported to support these cannot be validly extrapolated to humans, and the doses at which the studies have been performed are significantly higher than the levels to which humans are exposed.' (Kamrin MA, Int J Toxicol, 2007)
- Nevertheless, scientists have now begun to consider the concept of low dose exposure - although there is little consensus over what constitutes 'low dose'.
- 'Low dose' is taken to mean doses that are in the range of human exposure or doses below those traditionally tested in toxicological studies.
- Our natural endogenous hormones act at extremely low serum concentrations, typically in the picomolar to nanomolar range. So it makes sense that EDCs will have an effect in the same range.
- However, combination effects of low dose EDCs are never considered in risk assessment and regulatory standards (Kortenkamp A, 2014), largely because this presents theoretical and practical challenges.
- Furthermore, when 8 EDCs at levels below their 'No observed effect concentrations' (NOEC) were added together and their effects tested *in vitro*, there was a significant additive effect on steroidal oestrogens (Silva E, 2002; Rajapakse N, 2002), indicating that even where low dose single EDCs have no effect, a combination of low dose EDCs can have a profound effect.
- This is a truer reflection of real life, where we are exposed to many chemicals simultaneously, and suggests that it is body burden that causes the problem, not one exposure.



# EDCs: NON-MONOTONIC DOSE RESPONSES CURVES

- Discussion of 'low dose' often goes hand-in-hand with discussion of non-monotonic dose response curves (NMDRCs). These are non-linear dose response curves that changes sign. These are often U-shaped or inverted U-shaped, showing a bi- or multi-phasic response.
- Example of a NMDRC: Cells exposed to BPA showed a response only at intermediate doses, not high or low doses (Gualtieri AF, J Endocrinol Invest, 2010).
- Many scientists did not accept that a non-monotonic dose response existed because they could not envisage a mechanism for it (Vandenberg L, Endocr Rev, 2012).
- Also it meant that scientists and policy makers could continue making the assumption that one can extrapolate from high dose exposure effects to determine low dose effects.
- It makes sense that the dose response curve should be U-shaped because this is often the relationship between natural hormone concentrations and the number of bound receptors. And mechanisms for the effect have now been identified (Vandenberg L, Endocr Rev, 2012).
- Experimental data indicate that EDCs and hormones do not have 'No observed adverse effect levels' (NOAELs) or threshold doses and therefore no dose can ever be considered safe.



**A.** Linear responses, positive or inverse associations, allow for extrapolations from one dose to another.

**B.** Monotonic, nonlinear responses. The slope of the curve never changes sign, but it does change in value. Extrapolation only within the linear range.

**C.** Three different types of NMDRC: an inverted U-shaped, a U-shaped and a multiphasic curve. The slope of the curve changes sign one or more times. Extrapolation not possible.

**D.** Binary response, where one range of doses has no effect, then a threshold is met and all higher doses have the same effect.

Vandenberg L, Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev.* 2012; 33(3):378-455<sup>60</sup>



# EXAMPLES OF A TYPICAL REACTION TO A NON-MONOTONIC DOSE RESPONSE

1. 'Although we found associations between (phthalate) urinary concentrations and altered levels of FSH,

*the hormone concentrations did not change in the expected patterns.*

Therefore, it is unclear whether these associations represent physiologically relevant alterations in these hormones.' (Duty SM, Hum Reprod, 2005).

2. Faced with a non-linear dose response relationship, the US EPA concluded that 'lack of a (*linear*) dose-dependent response negated the importance of the effect'. (Hayes TB. 2002).

