

Endocrine disrupting pesticides

Many pesticides are now suspected of being endocrine disruptors - chemicals that can lead to an increase in birth defects, sexual abnormalities and reproductive failure. Gwynne Lyons of WWF-UK examines the current evidence and potential for adverse effects to occur in both wildlife and human populations.

Endocrine disrupting chemicals (EDCs) are substances that can cause adverse effects by interfering in some way with the body's hormones or chemical messengers. These substances are therefore called hormone disruptors or endocrine disruptors, as it is the endocrine glands that secrete the hormones.

Hormones play a crucial role in guiding normal cell differentiation in early life forms, and so exposure to endocrine disrupting substances in the egg or in the womb can alter the normal process of development. Mature animals can also be affected, but it is the developing organism that is especially vulnerable. Exposure at this sensitive time may cause effects that are not evident until later in life, such as effects on learning ability, behaviour, reproduction and increased susceptibility to cancer and other diseases.

Few official lists of suspected endocrine disruptors have been published. Table 1 details the pesticides that have been identified as potential endocrine disruptors in the list produced under the auspices of the Oslo and Paris Commissions (OSPAR). Table 2 details additional pesticides which might be endocrine disruptors and which feature on the World Wide Fund for Nature (WWF) list of chemicals in the environment reported to have reproductive and/or endocrine disrupting effects. However, for some of these substances, without further detailed investigation of their mode of action, it is not known whether their reproductive effects are actually the consequence of endocrine disruption. Apart from the pesticides documented in these two tables, others suspected of having endocrine effects include: metam sodium, methylbromide, carbendazim, prochloraz, dibromoethane (EDB), propanil, iprodione, thiram, diuron, diazinon and fenthion. These pesticides were amongst the 116 substances on which information was examined by EU experts, brought together by the European Commission in September 1999 for the purpose of drawing up a list of endocrine disrupting substances.

Effects of EDCs

The effects that can be seen in an organism exposed to an endocrine disrupting chemical (EDC) depend on which hormone system is targeted. For example, if an organism is exposed to sex hormone disrupting pesticides in the womb, then the sort of effects that may be evident include effects on sexual behaviour, structural deformities of the reproductive tract, including intersex type conditions and undescended testes, deficits in sperm counts, and effects on sex ratios. However, if the primary action is on the thyroid hormones, then as these hormones are responsible for metabolism and normal brain development, exposure in the womb may cause effects on intelligence and growth. Laboratory tests have confirmed that endocrine disrupting chemicals do indeed cause such effects in exposed animals, but all the effects listed above have also been noted in wildlife or humans heavily exposed to endocrine disrupting pesticides or industrial chemicals.

Some endocrine disruptors may exert their action by interfering with the brain's release of hormones, which in turn regulate the production of other hormones that control the growth and the activity of many other endocrine glands. Indeed, the pituitary has been termed the conductor of the endocrine orchestra, and pollutants that cause the pituitary region in the brain to malfunction may therefore have multiple effects.

Pesticides that are POPs

There is particular concern about endocrine disrupting pesticides that are lipophilic (fat loving), resistant to metabolism, and able to bioconcentrate up the food chain. This is because these substances become stored in body fats and can be transferred to the developing offspring via the placenta or via the egg. Predator animals (and humans) feeding

at the top of the food chain are at increased risk, particularly mammals because during breast feeding contaminants are again mobilised and transferred to the new born infant. Marine mammals may be most vulnerable, because not only do they carry large amounts of body fat, but also the oceans are the final sink for many persistent pollutants.

Some persistent pollutants, including several pesticides, are carried in air and in water over several hundred miles, and so even wildlife and people living far away from where these substances are used are under significant threat. Some areas are especially vulnerable because these substances are redistributed to the colder northern regions in a process termed 'global redistillation' or the grasshopper effect. This transboundary nature of pollution has led to the negotiation of a global agreement to control persistent organic pollutants (POPs), which is due to be finalised in 2001. The United Nations Environment Programme Convention on POPs will initially focus on 12 substances, including the following pesticides: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, HCB, mirex, and toxaphene. Public interest coalitions such as the International POPs Elimination Network (IPEN), the Pesticides Action Network and WWF are pushing for the production and use of these POPs to be eliminated as soon as possible.

DDT is, however, still used in several tropical countries. The challenge is for the global community to find other substances and regimes that are equally as efficacious in controlling malaria, and in this, WWF is certainly playing an active part. Unfortunately, even where it is now banned, exposure to DDT can still arise from a number of sources including: mobilisation of existing body burdens; from continued illegal usage; from sites contaminated in the past; and from continued usage elsewhere.

Mechanisms of action

General

Endocrine disruptors can exert their effects in many ways. They can either bind to the hormone's receptor and mimic the hormone, or block the action of the hormone. Alternatively, they can stimulate or inhibit the enzymes responsible for the synthesis or clearance of a hormone, and thereby give rise to an increased or decreased action of the hormone.

In general, with regard to endocrine disruptors, concern is mostly focused on those substances that cause endocrine mediated adverse effects at exposure levels lower than those which cause other adverse effects.

Sex hormone disruptors

The main hormone which gives rise to female characteristics is oestrogen, and the hormone mainly responsible for predominantly masculine characteristics is androgen. However, both sexes have both these hormones, although the levels of oestrogen are higher in females and androgens are higher in males.

Many pesticides have now been found to have oestrogenic or anti-androgenic activity, and some bind to the androgen or oestrogen receptors. Those which have been found to bind to the oestrogen receptor include: ortho-phenylphenol, DDT and metabolites (although the anti-androgenic properties of p'p'DDE may be of greater importance); methoxychlor; chlordane; dieldrin, endosulfan; 1-hydroxy-chlordane (a metabolite of chlordane); and toxaphene.

Some of these can induce oestrogenic effects at relatively low levels. For example, administration of methoxychlor to the new born rat at a dose level of 0.5 µg per day caused accelerated puberty and accelerated loss of fertility. Similarly, new born female rats injected with 1 mg per day of o'p-DDT on days 2-4 after birth had early onset of puberty and accelerated loss of fertility. Even doses as low as 1µg/day of either of these substances, given to pregnant female mice on days 11-17 of pregnancy, causes effects on the territorial behaviour of male offspring. However, DDE induced eggshell thinning, one of the most well known effects noted in wildlife, is now not thought to result from DDE binding to a sex hormone receptor.

Anti-androgenic pesticides that bind to the androgen receptor include: the dicarboximide fungicides, vinclozolin and procymidone; p'p'DDE; certain pyrethroids; and the herbicide

linuron. Researchers have evaluated the potency of the following pyrethroids in terms of their interaction with androgen binding sites, and in descending order this was: fenvalerate > phenothrin > fluvalinate > permethrin > resmethrin. In the case of vinclozolin, it is the metabolites that are active anti-androgens.

The dose levels at which effects are noted are fairly low. For example, at a vinclozolin dose level of 3 mg/kg/day, male rats exposed in the womb were feminised, in that abnormal numbers of nipples were seen. Similarly, at a dose level of 25 mg/kg/day given from the fourteenth day of pregnancy to three days after birth, procymidone caused intersex characteristics in male rats, but these workers did not determine a no-observed adverse effect level. Linuron has a similar structure as the pharmaceutical anti-androgen, flutamide, and at a dose level of 40 mg/kg/day from weaning through puberty, it reduced seminal vesicle weights in male rats and delayed puberty.

Pesticides which affect steroid synthesis and metabolism

Numerous pesticides have been reported to affect hormone synthesis and/or metabolism. These include: the imidazole pesticides (such as propiconazole, epoxiconazole and ketoconazole); fenarimol; TBT; and several organochlorine pesticides.

Ketoconazole, for example, has been found to block steroid synthesis, and in pregnant rats exposed to 25mg/kg/day from the fourteenth day of pregnancy, giving birth was delayed and a reduced number of pups survived. The authors suggested that ketoconazole inhibited the synthesis of oestradiol near term, possibly by inhibiting aromatase activity. Another pesticide, fenarimol, is known to inhibit aromatase activity, and this has also been shown to delay birth. TBT is also believed to act by inhibiting aromatase, as it appears to act by blocking the conversion of testosterone to oestradiol. It therefore has well-known androgenic activity in molluscs, and for example, it can cause female dog whelk to grow penises (imposex) at concentrations as low as 2.5 nanogram per litre.

Thyroid hormone disruptors

Other pesticides can act on the thyroid. For example, the following substances can affect thyroid hormone levels: amitrole; ioxynil; and the dithiocarbamates (such as maneb, mancozeb, and zineb). Amitrole (or aminotriazole) appears to interfere with thyroid hormone synthesis and can cause cancer of the thyroid. It is a triazine herbicide, with a no observed adverse effect level for thyroid hyperplasia of 2mg/kg in the diet of rats. Similarly, alachlor, an aniline-type herbicide, is associated with thyroid follicular tumours in rats, and is believed to be an endocrine disruptor.

Effects on brain

With regard to pesticides that act on the brain, both organophosphate and the insecticidal carbamate pesticides can reduce acetylcholinesterase (enzyme) activity, and hence block nerve impulses. This effect may be linked to the suppression of the brain's release of hormones that stimulate the gonads (the gonadotrophic hormones, which are follicle stimulating hormone (FSH) and leutinizing hormone (LH)).

Some organophosphates have been associated with decreased egg production and reduced serum luteinizing hormone (LH) in birds, and similarly, carbamates have been associated with a reduced number of eggs. Also, in the males of several animal species, certain organophosphates and carbamates have been linked with effects on sperm. Some organophosphate pesticides have been suggested to cause abnormal menses, amenorrhea, and early menopause, and again these effects have been linked with a perturbation of LH release from the pituitary. Likewise, exposure to carbaryl has been associated with adverse effects on human semen.

Aldicarb, an extremely toxic systemic carbamate insecticide, is also suspected of being an endocrine disruptor. When given to female pregnant rats at low levels of 1-100mg/kg, it has been shown to depress acetylcholinesterase activity more in the foetus than in the mother. It has also been suggested that there may be a link between low level exposure and effects on the immune system.

Assessing mode of action

The processes involved are much more complicated than this summary might suggest, and for example, not only are there many feedback mechanisms, but also the nervous, endocrine and immune systems are interconnected.

Our knowledge of hormonal actions and receptor sites is also far from complete, and two receptors for oestrogen have recently been identified. In addition, apart from the sex hormones and thyroid hormones there are many other hormones involved, not least including retinoids, progestins, and corticosteroids. Furthermore, apart from hormone messengers, there are many other signalling processes involved. This situation is further complicated by the fact that although chemicals can be shown to bind to certain receptors in test tube experiments, it is sometimes difficult to elucidate whether the adverse effects that they cause in animals are actually mediated primarily by the endocrine system.

Exposures to ECDs

Wildlife will be especially vulnerable to the endocrine disrupting effects of pesticides, because these chemicals are deliberately released into the environment. Effects linked to endocrine disruption have been noted in invertebrates, reptiles, fish, birds, and mammals living in polluted areas, but although most are linked to exposure to organochlorines, it is always difficult to tie down particular causal agents with any certainty. Humans exposed occupationally are also at increased risk, and there are studies linking exposure to pesticides at work to impotence, reduced sperm counts, increased time to pregnancy, and increased rates of birth defects in offspring. Similarly, in the Yaqui children in Mexico, who are highly exposed to pesticides, developmental effects have been reported, and in women highly exposed to DDT, shortened lactation has been noted.

The general public are exposed from residues in fruit and vegetables, and from contaminated meat, fish, and dairy produce, due to the build up of persistent and bioaccumulating pesticides in the food chain. Some hormone disrupting pesticides, such as linuron and atrazine may also be found occasionally in drinking water.

Apart from the active ingredient, nonyl phenol ethoxylates may be used as the surfactant in pesticides, and these can break down to nonyl phenol, an oestrogen mimic.

However, it is not only the effects due to any one particular spraying operation which give rise to concern, the main worry is with the potential interactive effects of the numerous hormone disrupting substances to which humans and wildlife are now exposed. Undertaking risk assessment on single substances will not replicate the real world situation. It could certainly be envisaged that exposure to oestrogen mimicking substances, anti-androgenic substances, substances which inhibit the formation of steroids, and substances which increase their clearance, could all give rise to an enhanced de-masculinising effect.

Recommendations for controls

The possible additive or synergistic effects, and the need to review no observed effects levels (NOELs) with regard to endocrine effects, certainly provide a powerful argument for the implementation of larger safety factors if 'acceptable levels' of exposure to hormone disrupting substances are to be defined. This approach assumes, of course, that even for hormone disruptors acting as developmental toxins there is some biological threshold, and it would certainly be wiser to aim to eliminate exposures. Behavioural effects have been noted at low levels of exposure, and particularly taking into account the range of species upon which an ecosystem depends, it is doubtful if toxicity tests could be undertaken to pick up on all such potential effects, which could nevertheless have profound population level effects. Therefore, WWF UK believes that the goal should be to eliminate exposures to endocrine disrupting substances where possible. In particular, there should be a rapid move away from the use of endocrine disrupting pesticides that are also persistent and/or bioaccumulative.

Table 1: Evidence of endocrine disrupting effects

Pesticide and usage	Human exposure routes	vth	vh	vtw	vw
Atrazine A herbicide used on non-crop land and in agriculture, including, for example, weed control in maize (sweetcorn). Banned for non-crop use in UK. P	Has been found in drinking water on occasion	y	y*		y
Beta-HCH May be found as an impurity in lindane. Also formed as a by-product in the manufacture of lindane. UN ECE POP = P + B + T	In the past, high levels have been found in rabbit imported from China, and it has also been found in meat and fish and butter oil. Also found in breast milk, - and still found in 1996/97 UK samples, although levels have decreased.		y		y
Chlordane Mainly used to control termites, and on home lawns and gardens. Now widely banned. UNEP POP = P + B + T	On occasion, has been found in breast milk, as has oxychlordane the stable metabolite. Due to atmospheric transport, Inuit women tend to have a diet highly contaminated with chlordane.	y	y	y	
Chlordecone (Kepone) Used to control insects on crops, including bananas and tobacco. Has also been used against ants and cockroach. UN ECE POP = P + B + T	Exposure has occurred due to eating contaminated fish and animals. Has also been found in clams, and in breast milk.	y*	y*	y	y
DDT Banned in all countries for use in agriculture. Still used in disease vector control (eg. Malaria) - recommended for indoor spraying only. UNEP POP = P + B + T	Residues found in beef (from Brazil and Zimbabwe). In the past, found in rabbit (from China) and in cow's milk and butter. Recently found in fish (tuna) and imported lamb's liver. Found in breast milk-and metabolites still found in UK samples, although declined since banned.	y*	y*		y
Dicofol A non-systemic organochlorine acaricide. Usage includes on cucumber, tomatoes, lettuce, ornamentals, hops, apples and strawberries. Can be contaminated with alpha-CI-DDT. In EU, dicofol is not permitted if it contains less than 78% of pp dicofol or more than 1g/kg DDT and related compounds.	May be found in apples and lemon products. Been found as a contaminant in human body fat in US surveys in the 1980s.				y

<p>Dieldrin Has been used as a sheep dip and in wood treatment. No longer produced. (NB. aldrin can break down to dieldrin). UNEP POP = P + B + T</p>	<p>A 1992 survey in the UK found very high levels in eels. Also in 1993 it was found in samples of UK cow's milk and butter oil. Dieldrin has been found in breast milk, and is still found in some UK samples, although levels have declined since usage was banned.</p>	y*	y*	y	
<p>Endosulfan A contact and ingested organochlorine insecticide and acaricide, usage includes on hops, rape, several soft fruits, and on ornamentals. Widely used in developing countries. P + B</p>	<p>Residues have been found in 1998 UK samples of peas, mange tout, tomatoes, and soft fruit, such as plums and blackcurrant. In the early 1990s, endosulfan was found in baby foods in the US.</p>	y	y*	y	
<p>Hexachlorobenzene HCB Was used as a seed treatment and as a fungicide, but is now banned in several countries. Found as a contaminant of quintozene, tecnazene, chlorothalonil, picloram, and chlorthal-dimethyl (dacthal or DCPA). UNEP POP = P + B+ T</p>	<p>Found in rabbit from China. Has also been found in earlier surveys of eels, meat, cow's milk and cheese. HCB has also been found in breast milk, and still found in some UK samples although levels have declined.</p>		y		
<p>Lindane (Gamma-HCH) A contact, ingested, and fumigant organochlorine insecticide, used on many crops including sugar beet and oil seed rape. Also used as a timber treatment, and in the home, for head lice. (now banned for head lice in the UK) UNECE POP = P + B + T</p>	<p>A 1998 survey found lindane in 75% of samples of chocolate with a high cocoa butter content. Also found in 1998 samples of cereal grains, and mushrooms and in earlier surveys in fish, meat, butter, cheese, flour, bread and some vegetables, such as onions. Has also been found in cow's milk in the UK, although not detected in the latest survey in 1998. Still detected in some UK breast milk samples.</p>	y*	y*		y
<p>Methoxychlor Insecticide used on fruits, vegetables, forage crops and livestock. P + B</p>			y*		
<p>Pyrethroids Insecticides widely used in agriculture. Permethrin also used for timber treatment. The OSPAR document does not specify which are believed to be EDCs.</p>	<p>When used for timber treatment, exposure may occur via inhalation. Some have been found in rivers.</p>	y			
<p>Toxaphene A mixture which was used as an insecticide, mostly on cotton and other</p>	<p>Due to atmospheric transport, Inuit women tend to have a diet highly contaminated with</p>	y	y	y	

crops. Used to control ticks and mites in livestock. Now widely banned. UNEP POP = P + B + T	toxaphene compounds. High levels found in marine mammals. Also found in Arctic and Baltic fish, such as salmon. In the 1980s was found in a survey of Swedish breast milk.				
Triazines Atrazine, simazine, cyanazine, amitrole and prometryn are triazine herbicides. The OSPAR document does not specify which are believed to be EDCs.	Like atrazine, simazine has occasionally been found in drinking water.	y			
Tributyl tin Used as a biocide in antifouling paints, wood and material preservatives, and in plastic, paints and insulants. Has also been used in duvets.	Found in fish, and especially high levels were found in Baltic fish. Has been found in food cooked on baking parchment. Human exposure does occur as butyltins have been found in human liver.				y
Vinclozolin A dicarboximide fungicide used on rape, beans, peas, turf and apple blossom. In UK, some illegal usage on winter lettuce. P + B	Residues found in UK lettuce in 1997 samples due to illegal usage. Also found in 1998 samples of kiwi fruit, peaches/nectarines, and tomatoes, and earlier samples of peas, peppers, orange, beans, cress, garlic, grape juice, salmon, and sultanas. A related anti-androgenic fungicide, procymidone has similarly been found in UK lettuce, and also in aubergines, peas, pears, wine and tomatoes.	y	y		

Notes to Table: P= Persistent B= Bioaccumulative (These symbols are used as found in the OSPAR document); UNEP POPs are chemicals designated for inclusion in the Convention and are therefore defined as P, B and T (toxic).; UN ECE POPs are chemicals covered by the UN ECE Protocol on POPs and are therefore subject to atmospheric transport and are also defined as P + B + T (all UNEP POPs are also UN ECE POPs).

*The y indicates endocrine disrupting effects are as found in the OSPAR document DIFF 99/3/20-E Rev. 1(L). They are not intended to be exhaustive, but the * is used as found in the OSPAR document and denote 'and others'. Some references may be incorrect, but apart from those detailed for the pyrethroids, they are as stated in the OSPAR document.*

vth = In vitro (relevant to humans)

vh = in vivo (relevant to humans)

vtw = in vitro (relevant to wildlife)

vw = in vivo (relevant to wildlife)

Table 2: WWF list of pesticides in the environment reported to have reproductive and/or endocrine disrupting effects

Herbicides

2,4-D, 2,4,5-T, acetochlor, alachlor, amitrole, atrazine, bromacil, bromoxynil, cyanazine, DCPA (dacthal), ethiozin, glufosinate-ammonium, ioxynil, linuron, metribuzin, molinate, nitrofen, oryzalin, oxyacetamide/fluthamide (FOE 5043), paraquat, pendimethalin, picloram, proflam, pronamide, simazine, terbutryn, thiazopyr, trichlorobenzene, trifluralin

Fungicides

benomyl, etridiazole, fenarimol, fenbuconazole, hexachlorobenzene, mancozeb, maneb, metiram, nabam, penachloronitrobenzene, pentachlorophenol, triadimefon, tributyltin, vinclozolin, zineb, ziram

Insecticides

aldicarb, aldrin, bifenthrin, carbaryl, carbofuran, chlordane, chlordecone, chlorfentezine, 8-cyhalothrin, DDT and metabolites DDE, DDD, deltamethrin, dicofol, dieldrin, dimethoate, dinitrophenol, endosulfan (a and b), endrin, ethofenprox, fenitrothion, fenvalerate, fipronil, a-HCH, heptachlor and H-epoxide, lindane (g-HCH), malathion, methomyl, methoxychlor, mirex, oxychlordane, parathion (methylparathion), photomirex, pyrethrins, synthetic pyrethroids, ronnel (fenchlorfos), toxaphene, transnonachlor

Nematicide

DBCP

Rodenticide

n-2-fluorenylacetamide

Sources:

1. Colborn T, 1998, *Endocrine disruption from environmental toxicant*. In Rom W N (ed) *Environmental and Occupational Medicine, Third edition*, Lippincott-Raven Publishers, Philadelphia.
 2. Brucker-Davis F, 1998, *Effects of environmental synthetic chemicals on thyroid function*, *Thyroid* 8(9), p827-856.
 3. Short P, Colborn T, 1999, *Pesticide use in the US and policy implications: a focus on herbicides*, *Toxicol Ind Health* 15(1/2), p240-275.
- (See WWF Canada's site on the internet for list and references)

Vir: <http://www.pan-uk.org/pestnews/actives/endocrin.htm>