CHEM Trust

Protecting humans and wildlife from harmful chemicals

EFFECTS OF POLLUTANTS ON THE REPRODUCTIVE HEALTH OF MALE VERTEBRATE WILDLIFE - MALES UNDER THREAT

A CHEM Trust report by Gwynne Lyons

CHEM Trust's aim is to protect humans and wildlife from harmful chemicals. Based in the UK, it was set up in 2007 to take over the mantle of WWF-UK's work on toxic chemicals. CHEM Trust's particular concerns relate to chemicals with hormone disrupting properties, persistent chemicals that accumulate in organisms, the cocktail effect and the detrimental role of chemical exposures during development in the womb and in early life. CHEM Trust passionately believes in the conservation of biodiversity and in the importance of wildlife protection. Furthermore, monitoring wildlife populations can provide vital insights into contaminant-related threats to human health, the protection of which is of paramount importance.

Both wildlife and humans are at risk from pollutants in the environment. CHEM Trust is working towards a time when chemicals play no part in causing impaired reproduction, deformities, disease, deficits in brain function, or other adverse health effects. Human exposure to some undesirable chemicals may arise from contamination of the food chain and from the use and disposal of many everyday products such as TVs, computers, cars, construction materials, toys, toiletries and cosmetics.

CHEM Trust is committed to engaging with all parties, including regulatory authorities, scientists and medical professionals to increase informed dialogue on the harmful role of some chemicals. By so doing, CHEM Trust aims to secure agreement on the need for better controls over certain chemicals, and thereby to prevent disease and protect both humans and wildlife.

contents

Abbreviations and Technical Terms.

a BHC	alpha benzene hexachloride (related to Lindane insecticide) alternatively called alpha hexachlorocyclohexane.
anti-androgenic	a hormone disruptor which works against the male hormone, androgen.
cryptorchidism	undescended testes (bilateral refers to both testes, and uni-lateral cryptorchidism means one testis is undescended).
EDCs	endocrine or hormone disrupting chemicals. The term 'endocrine disrupting chemicals' is interchangeable with the term 'hormone disrupting chemicals' or 'hormone disruptors'. Hormone disruptors are substances, not naturally found in the body, that interfere with the production, release, transport, metabolism, binding, action or elimination of the body's natural hormones, which function as chemical messengers.
Dioxins	polychlorinated dibenzodioxins (PCDDs), combustion products.
DDT	dichloro diphenyl trichloroethane, an insecticide.
DDE	dichloro diphenyl dichloroethylene, a contaminant or breakdown product of DDT insecticide.
DNA	deoxyribonucleic acid.
Furans	polychlorinated dibenzofurans (PCDFs), combustion products.
HCB	hexachlorobenzene, a fungicide.
OCs	organochlorine chemicals.
Oestrogenic	hormone disruptor mimicking the female hormone, oestrogen.
Ovo-testes	eggs developing in the testes / intersex features.
PCBs	polychlorinated biphenyls, a now banned persistent pollutant which was used principally in electrical equipment.
TDS	testicular dysgenesis syndrome.
TSH	thyroid stimulating hormone.
VTG	vitellogenin, the egg yolk precursor protein made by females.

section 1
summary and overview

This paper provides a review of the reported effects on the reproductive health of male vertebrate wildlife, which are known or suspected to be associated with pollutants. Males of species from each of the main classes of animals in the vertebrate sub-phylum (including bony fish, amphibians, reptiles, birds, and mammals) have been affected by chemicals in the environment, particularly chemicals with hormone disrupting properties. Man made chemicals that can disrupt the male and/or female sex hormone may adversely affect the ability of an organism to reproduce, although chemicals which affect reproduction by other mechanisms are also of concern.

All vertebrates have similar sex hormone receptors, which have been conserved in evolution. Therefore, observations in one vertebrate wildlife species, may serve to highlight pollution issues of concern for other vertebrates, including humans. Indeed, given the widespread presence of endocrine disrupting chemicals in the environment, effects are likely to be occurring in more species than those currently reported. Endocrine disrupting chemicals (EDCs) de-rail the body's chemical messenger system, the hormones, and therefore this term is used interchangeably with the term 'hormone disruptors'. Auxiliary signalling chemicals such as enzymes, growth factors, and so forth, may also be disrupted. There is much "cross talk" in the body, and, for example, pollutant related disruption of brain neurochemistry can be an early step in reproductive impairment (Basu and Head,2008). The mounting concern is such that between 1998 -2007 the European Commission invested 161 million Euros into research into the phenomenon of endocrine disruption.

Section 2 summarises the effects reported in male vertebrate wildlife. These include altered hormone levels, reduced number of sperm, genital deformities and deformities of other structures under sex hormonal influence. Many of these reported effects are known or suggested to be due to exposure to EDCs in the environment. Feminization of the males of numerous vertebrate species is now a widespread occurrence, with many males of egg laying vertebrate found to be abnormally producing

the egg yolk precursor protein, vitellogenin. Vitellogenin (VTG) is synthesized by the liver of non-mammalian vertebrates and induced in response to oestrogen. A decrease in male sex hormone, or in the ratio of the male:female sex hormones can lead to weak male secondary sex characteristics including intersex reproductive organs (part female ovary, part male testis), small penis, ineffective mating behaviour, and possibly low fertility. This review also highlights some species where reduced reproduction has been noted, but this may be due to effects of contaminants on the female of the species, rather than the male. Moreover, the mechanisms of action by which some of the effects occur are not known with certainty.

In male vertebrate wildlife the following effects, which are known or suspected to be caused by pollutants, have been reported.

In fish: abnormal secretion in males of VTG; altered spermatogenesis; eggs developing in testes (ovo-testes/intersex); intersex genital apparatus; and poor reproductive success.

In amphibians: abnormal production of VTG by males and ovo-testes/intersex features.

In reptiles: abnormal production of VTG by males: sex hormone disruption; ovo-testes; smaller phallus in alligators and shorter estimated penis length in turtles; decreased hatching; and decreased post hatch survival.

In birds: abnormal VTG production in male birds; deformities of the reproductive tract; embryonic mortality; reduced reproductive success including egg-shell thinning; and poor parenting behaviour.

Effects in the males of numerous mammalian species have been reported, and include the following.
In rodents: reduced sperm; reduced testes weight; and reduced reproduction.
In otters and/or mink: reduced baculum (penile bone) length; smaller testes; and impaired reproduction.
In seals and/or sea lions: impaired reproduction (including implantation failure, sterility, abortion, premature pupping).
In cetaceans: reduced testosterone levels; impaired reproduction; and hermaphrodite organs.
In polar bears: intersex features and deformed genitals; reduced testes and baculum length; low testosterone levels in adult males; and reduced cub survival.
In black bears: undescended testes.
In Florida panther: abnormal sperm and low sperm density; undescended testes; and altered hormone levels.
In deer: antler deformities; undescended testes; and testicular abnormalities, including cells predictive of testicular cancer.
In eland (an antelope): abnormal testes, including impaired spermatogenesis.

These findings are tabulated in Table 1, and are discussed in more detail in Section 2. Taken together, it can be seen that feminisation or de-masculinisation of males is widespread.

Section 3 provides an overview of some of the findings in wildlife living in polluted environments. This section also highlights the difficulties of identifying which particular pollutants are to blame for such effects, and summarises the concern about effects due to simultaneous exposure to more than one chemical, the so called 'mixture effect'. Several oestrogenic and anti-androgenic chemicals that have been found in polluted rivers and lakes are noted, as is the anti-androgenic activity found in discharges from UK sewage works. Furthermore, this section highlights that concern for the long-term health of wildlife populations and humans is enhanced because several laboratory studies have suggested that disorders, such as deficits in sperm production, can be passed on to subsequent generations, who themselves have not been exposed. Such effects are termed transgenerational.

Section 4 draws conclusions and recommendations. It underlines the similarities of the reported effects in male vertebrate wildlife, and therefore notes the concern for human male reproduction. Conclusions are also reached regarding the need for tighter regulation of EDCs in order to reduce exposures. In addition, the need for ongoing monitoring of wildlife is also highlighted, as is the need for more research to understand the long-term implications of chemical exposures for life on earth.

section 1
summary and overview
(cont)

Table 1: Effects reported in wildlife which are known or suggested to be linked to chemical contaminants

		Reduced reproduction	Intersex / Abnormal Testes	Deformities of sex linked structure / reduced phallus/ baculum	VTG in male	Other Sex linked effect
FISH	Fish	Y	Y	Y	Y	
AMPHIBIAN	Frogs/Toads	Y	Y		Y	Reduced no. of nuptial pads in males
REPTILE	Alligator	Y	Y	Y		
	Turtle	Y	Y	Y	Y	
BIRDS	BIRDS	Y	Y		Y	Egg shell thinning
MAMMALS	Rodent	Y	Y			
	Otter	Y	Y	Y		
	Mink	Y		Y		
	Seal / Sea Lion	Y				
	Whales (Cetaceans)	Y	Y			
	Polar Bears	Y	Y	Y		
	Black/Brown Bears		Y			
	Panther	Y	Y			
	Deer	Y	Y			Deformed antlers in males
	Eland		Y			

Y = Effect reported and known or suggested to be linked to contaminants

section 2
pollutant-related effects reported in male vertebrate wildlife and effects on reproduction

Many wildlife species are now reported to be affected by pollutants, and similarities can be seen in the effects recorded. The target sites which are the focus of this review include male developmental pathways. It is clear that structural intersex features, including effects on the male reproductive tract, result from exposure before birth. On the other hand, abnormal secretion of the egg yolk precursor protein, VTG, in male fish, birds, and reptiles, can result from later adult-life exposure to feminising pollutants. VTG is normally produced in females, and when found in males in elevated concentrations it confirms the presence of sex hormone disrupting contaminants in the environment, and indicates feminisation of the male. Reduced reproduction has also been included, although it may result from female or male reproductive impairment, or from lack of viability of the offspring.

Field studies of wildlife are expensive and time consuming to conduct and there is therefore a paucity of information on most species. Apart from studies in highly polluted areas, most of the data on wildlife come from species hunted for food, particularly fish.

Table 1 illustrates that defects linked to male reproductive development appear to be common to wildlife species from each of the classes of animals that make up the vertebrate sub phylum. It shows that contaminants are affecting the reproductive health of males of many species. Feminisation or de-masculinisation of males is widespread.

2.1
fish

Fish may be particularly affected by pollutants, because their exposure is not only via the diet, but also via the gills and skin. The physical chemical characteristics of many EDCs, especially their lipophilicity ("fat-loving" properties) also favour their movement from the surrounding water into biological tissues.

In fish, the following effects have been particularly noted: abnormal secretion of VTG in males; altered spermatogenesis; eggs developing in testes (ovo-testes/intersex); intersex genital apparatus; and poor reproductive success.

VTG, the precursor of the egg yolk protein, is normally not detectable in male fish, or is only present at very low levels. Therefore, detection of elevated levels in male fish is abnormal, and is an excellent biomarker of exposure to oestrogenic EDCs. Furthermore, VTG induction is generally accompanied by various degrees of reproductive interference at similar or lower ambient oestrogen concentrations. This means that it can be a marker for a number of adverse effects (for review see Matthiessen,2003)

If reproduction in males is compromised, and fewer males contribute to the next generation, this would not necessarily affect the population in the short term. Population levels may largely depend on the number of female offspring that result from the average female's lifetime reproductive activity (Gurney,2006). Nevertheless, a considerable proportion of breeding males are believed to be necessary in order to sustain a genetically viable population in the long term (IEH,2004). The following examples represent some of the studies showing a link between exposure to EDCs and effects in fish from the *Osteichthyes* class (the bony fish).

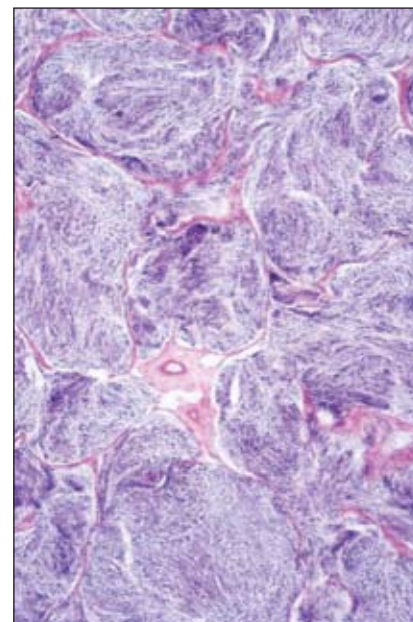## Abnormal Production of Vitellogenin (VTG) in Male Fish

Studies in UK freshwaters were the first to report the phenomenon of VTG production in male fish (Purdom et al.,1994; Harries et al.,1996). Similarly, subsequent UK studies by Lye and co-workers (1997;1998) were the first to report VTG induction and testicular abnormalities in a marine fish, the flounder (*Platichthys flesus*). In many UK fresh waters downstream of sewage treatment works it seems that a large part of the oestrogenic component is derived from the natural female hormones (oestrone and oestradiol-17b) and the contraceptive pill (ethinyl oestradiol) excreted in sewage (Jobling and Tyler,2003). However, in some UK rivers, industrial chemicals, such as nonylphenol, have also been implicated as a causal factor in VTG production (Thorpe et al.,2001; Lye et al.,1999). Similarly, in the Mediterranean, some researchers suggest that oestrogen mimicking organochlorine contaminants may play a role (Fossi et al.,2004).

VTG production in several wild male freshwater fish species has now been reported in many places worldwide including Europe, North America, Australia, Japan and Africa. In the Republic of Benin in Africa, for example, VTG in male tilapia (*Sarotherodon melanotheron*) has been associated with organochlorine pesticide contamination in the Ouémé River (Okoumassoun et al.,2002). Similarly, VTG production in male marine fish has also now been

reported in many species and many countries worldwide, including: cod (*Gadus morhua*) from the North Sea (Scott et al.,2006); dab (*Limanda limanda)* from the North Sea, Irish Sea and English Channel (Scott et al.,2007); flounder from UK estuaries (*Platichthys flesus*) (Kirby et al.,2004); flounder from Denmark; flounder from a Dutch harbour and a Dutch offshore spawning ground; sole (*Pleuronectes yokohamae*) from Japan; grey mullet (*Mugil cephalus*) from Osaka Bay in Japan; sole (*Parophrys vetulus*) from Puget Sound, USA (for review see Matthiessen,2003); and Mediterranean swordfish (*Xiphias gladius*) from the Straits of Messina near Sicily, where VTG induction was seen at very high levels (Fossi et al.,2004).

Flounder - normal testis tissue seen through microscope.
[Photo kindly provided by ©Mark Kirby, CEFAS Lowestoft]

Male flounder and other marine fish, includng cod, dab, sole, grey mullet and swordfish have been found to be abnormally making the female egg yolk protein
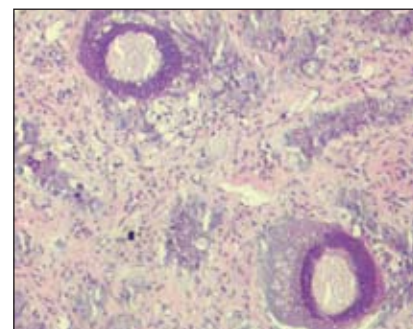
Flounder - Ovotestis. The circles are eggs in the testis. Up to a fifth of male flounder from certain sites in some UK estuaries are intersex.
[Photo kindly provided by ©Mark Kirby, CEFAS Lowestoft]

2.1
fish
(cont)

Intersex in Fish

The presence of intersex or ovo-testis (ie. primary or secondary oocytes (eggs) abnormally present in the testicular tissue of the male) is now a frequently reported phenomenon in fish. This disrupted gonad development is almost certainly linked to endocrine disruption caused by exposure to hormone disrupting compounds. It can be induced experimentally through exposure at the larval stage, but not by exposure of the adult fish. Male fish with intersex organs typically produce fewer motile sperm than those with normal testes. Intersex has been reported to varying degrees, in (up to 100% of) freshwater roach (*Rutilis rutilus*) at some locations on UK rivers (Jobling and Tyler,2003).

Freshwater fish species in which abnormal intersex has been reported include: roach; bream (*Abramis abramis*); chub (*Leuciscus cephalus*); gudgeon (*Gobio gobio*); barbel (*Barbus plebejus*); perch (*Perca fluviatilis*); white perch (*Morone Americana*) (Kavanagh et al.,2004); stickleback (*Gasterosteus aculeatus*); shovel-nosed sturgeon (*Scaphirhynchus platyorynchus*) (for review see Jobling and Tyler,2003); sharptooth catfish (*Clarias gariepinus*) (Barnhoorn et al.,2004); lake whitefish (*Coregonus clupeaformis*) (Michaelian et al.,2002) and smallmouth bass (*Micropterus dolomieu*) (Blazer et al.,2007).

The phenomenon of intersex in estuarine and marine fish in the UK appears to be less than in some UK freshwater fish, but it is not known whether this is due to species differences in response, higher exposures in the freshwater upstream, or the fact that breeding grounds for marine species are further offshore and therefore probably less contaminated. Nevertheless, in some very oestrogenically contaminated UK estuaries (Mersey, Tyne, Clyde and Forth) up to a fifth of the male flounder and blenny (eelpout) (*Zoarces viviparous*) in some locations show ovo-testes, whereas ovo-testes has not been seen in flounder from a relatively uncontaminated reference estuary, the Alde (for review see Matthiessen,2003).

Intersex is now known to be widespread. For example, apart from in the UK, it has been reported in fish from the Seine estuary in France (flounder); the southern Baltic in Germany (flounder);Tokyo (flounder); the Mediterranean (swordfish) (for review see Matthiessen,2003); South Africa (sharptooth catfish) (Barnhoorn et al.,2004); the Potomac river (small mouth bass) (Blazer et al.,2007); the St Lawrence river in Quebec (lake whitefish) (Michaelian et al.,2002); and in a polluted area of Lake Ontario, where 83% of male white perch collected in 1999-2000 had intersex features, which was an increase on the previous year (Kavanagh et al.,2004).

Deformities of Sex-Linked Structures in Fish

There are species differences in the response of fish to exposure to sex hormone disruptors. For example, sand gobies (*Pomatoschistus minutus and P.lozanoi*) from contaminated estuaries in the UK do not show either induction of VTG or intersex, but instead male fish exhibit deformed

and feminised urogenital papillae, which is the structure used by both sexes to deposit gametes (Matthiessen et al.,2002). In males, the papillae can be considered equivalent to the penis. This phenomenon in sand gobies has been termed morphologically intermediate papilla syndrome (MIPS), and was found in males from the UK Tees, Mersey and Clyde estuaries. This feminised condition was found in areas of known oestrogenic contamination, and laboratory experiments provided good evidence that it is probably caused by exposure to environmental endocrine disruptors. Nevertheless, in the wild the precise causal agent(s) were not identified.

Sand Goby
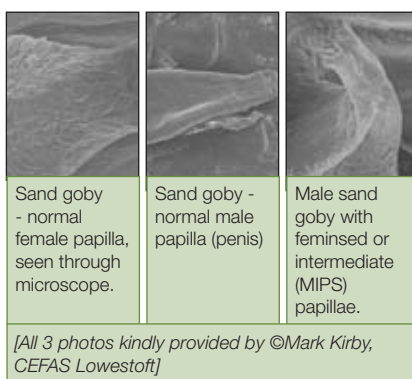
Sand goby - normal female papilla, seen through microscope.

Sand goby - normal male papilla (penis)

Male sand goby with feminsed or intermediate (MIPS) papillae.

[All 3 photos kindly provided by ©Mark Kirby, CEFAS Lowestoft]

In some UK estuaries and effluents, substances present with reported oestrogenic activity included the natural steroids, particularly 17b-oestradiol, and some synthetic man-made compounds, including nonylphenol and di-(2-ethylhexyl) phthalate (DEHP). In sediments, much higher oestrogenic activity was identified, and although the main contributors to this activity were

not identified, some of the minor contributors to the oestrogenic or feminising activity were reported to be nonylphenol, cinnarizine (an anti-histamine drug), and cholesa-4,6-dien-3-one (a natural cholesterol degradation product) (Allen et al.,2002).

Structural defects of the reproductive apparatus have also been noted in other fish species. In Florida USA, mosquito fish (*Gambusia holbrooki*) from the pesticide polluted Lake Apopka were compared with those from less polluted lakes. Male fish from the polluted Lake Apopka had slightly shorter gonopodia and fewer sperm cells per milligram weight of testis, when compared with the fish collected from Orange Lake and Lake Woodruff. The growth and development of the modified anal fin (the gonopodium) is a secondary sexual characteristic in males under the influence of testosterone, and is critical for sperm transfer. The authors concluded that sexual characteristics of relevance to male reproductive capacity are altered in the Lake Apopka mosquitofish population, and that anti-androgenic chemicals were a possible cause of the effects (Toft et al.,2003). Similarly, effects on the gonopodia of male mosquitofish taken from polluted wetlands in Western Australia have been noted (Game et al.,2006). In South Africa, abnormalities of the sexual papillae were found in male sharptooth catfish from a nature reserve in Pretoria. At four sampling sites in the reserve and in various matrices, several chemicals were present. These included residues of a-BHC, lindane, endrin, heptachlor epoxide, methoxychlor, DDT and metabolites, and octylphenol,

p-nonylphenol, diethylphthalate, dimethylphthalate, dibutylphthalate and DEHP (Bornman et al.,2007).

Poor Reproductive Success / Reduced Hatching in Fish
In Lake Ontario, contaminants were considered responsible for the loss of lake trout (*Salvelinus namaycush*) in the 1960s (Cook et al.,2003). Moreover, even after the extinction of this population of lake trout, and re-stocking, there was a lack of reproductive success, with reduced fry survival still occurring after 1980 (Cook et al.,2003). Cook and colleagues took sediment cores and were able to show that lake concentrations of dioxins and dioxin-like chemicals were probably to blame, because at the time of the crash, and for some years afterwards, these were sufficient to affect the breeding of these top predator fish.

Reduced spawning success or reduced hatching has been noted in several wild populations of marine fish, including: a DDT contaminated population of white croaker (*Genyonemus lineatus*) in California; a variety of flatfish species (*P.bilineatus and P.vetulus*) in the Puget Sound, USA; PCB contaminated Baltic flounder; PCB or DDE contaminated Baltic herring (*Clupea harengus*); and organochlorine contaminated Baltic cod (*Gadus morhua*) (for review see Matthiessen,2003).

2.2
amphibians

Many amphibian species worldwide are in decline, with an estimated one third now either threatened or extinct (IUCN,2008a). Habitat degradation is suggested to be the main contributory factor, but pollution may also play a role. Indeed, pesticides and industrial chemicals can be carried to remote areas far from the site of their release. In California's snow-capped Sierra Nevada, populations of frogs and toads have crashed, including the yellow-legged frogs (*Rana boylii and Rana muscosa*) and the California red-legged frog (*Rana aurora*), with some researchers suggesting that the high levels of pesticides transported in the air are responsible (Sparling et al.,2001). In male amphibians abnormal production of VTG and intersex features have been noted in some polluted locations. It is therefore speculated that some of the decline in amphibians may be due to effects on reproduction, although studies also suggest immune suppression due to chemical exposures may be a factor (Linzey et al.,2003; Christin et al.,2004; Fenoglio et al.,2005; Hayes et al.,2006).

Northern leopard frogs in the wild have been found with eggs in the testes.
[©iStockphoto.com-Steve Geer]

Reproductive System Defects, VTG Production and Intersex in Amphibians

Intersex features, linked to chemical exposure, have been seen in the wild in both frogs and toads, and feminisation of males may lead to less reproductive success.

Hayes and co-workers observed retarded gonadal development (*gonadal dysgenesis*) and oocytes in the testes of wild leopard frogs (Rana pipiens) collected from atrazine-contaminated sites across the USA (Hayes et al.,2003). They suggested that atrazine could be causing these effects in wild amphibian populations, and showed that atrazine exposure in the laboratory (at 0.1ppb) resulted in intersex characteristics in leopard frogs. In male leopard frogs exposed in the laboratory to 0.1ppb of atrazine, testicular oocytes were found in 29% of the males, and in some cases the oocytes were vitellogenic (Hayes, 2004). Furthermore, Hayes and colleagues (2003) have hypothesised that atrazine might induce aromatase, which converts testosterone to oestrogen, thereby increasing the production of endogenous oestrogen. However, another team of researchers have reported that higher concentrations of atrazine are needed to cause such effects (Carr et al.,2003). These workers subsequently concluded, after conducting outdoor experiments in tanks, that oocytes in the testes of the African clawed frog (*Xenopus laevis*) may be a natural phenomenon (Jooste et al.,2005). However, several other amphibian experts have expressed doubts about this latter finding (Renner,2005) and it may be a temperature related effect or perhaps due to other EDCs contaminating the water in the tanks.

In a study in Illinois, Reeder and co-workers (1998; 2005) concluded that several chemical contaminants including PAHs, PCBs, dioxins, furans, DDT and possibly atrazine, were likely to have contributed to the decline of cricket frogs (*Acris crepitans*). From studying museum collections they considered that the proportion of intersex individuals peaked during the period 1946 -1959. The genetic sex of these frogs was not confirmed, but it was believed that the ones with ovo-testes were males (Beasley,2008).

Male cane toads with intersex testes and lower testosterone have been found in Florida where pesticides, including atrazine and glyphosate, are used. *[©iStockphoto.com-Eric Delmar]*

Male cane toads (*Bufo marinus*) in the wild are also exhibiting signs of feminisation. McCoy and colleagues (2008) have studied populations in sugar cane fields in the Florida Everglades where pesticides, including glyphosate and atrazine, are used. These toads were compared with toads living in areas with less agriculture. The number and severity of feminised toads was greatest at sites with more agriculture, and the number of abnormalities and frequency of intersex gonads increased with agriculture in an application-dependent fashion.

Effects reported in the males, included intersex characteristics with both

testes and ovarian tissue present, female colouration called mottling, reduced nuptial pad number and smaller forearm widths. In the most heavily farmed areas almost 40% of the male toads were intersex, and had both testes and ovarian tissue.

Testosterone levels were also lower in the toads living in agricultural areas compared to those in cane toads from more suburban areas. Oestradiol levels were not affected, but due to the reduction in testosterone, the intersex toads from the agricultural areas also had a higher ratio of oestrogen to testosterone (McCoy et al.,2008).

Accumulation of the female egg yolk precursor protein, VTG, was seen in the Bidder's organ of around 20% of the male toads from agricultural areas. This is only known to occur after castration, and it is therefore considered that the testes are necessary to suppress accumulation of VTG in the Bidder's organ. Therefore, the authors concluded that their findings suggested that the testes of the affected toads were malfunctioning. Indeed, all the reported effects were considered suggestive of compromised testicular function.

Steroid hormone concentrations and secondary sexual traits correlate with reproductive activity and success, and the authors therefore maintain that the affected toads are likely to have reduced reproductive success, and that these reproductive abnormalities might certainly contribute to amphibian population declines in areas with agricultural contaminants (McCoy et al.,2008).

Other abnormalities and altered hormone levels in amphibians

A disorder characterised by an extra or malformed limb(s) has been reported in frogs, with some scientists suggesting that this may be linked to chemicals, UV exposure, trematode infection, acid rain, viruses, nitrates, or a combination of these (Kiesecker,2002; Gardiner et al.,2003; Ankley et al.,2004; Bridges et al.,2004).

A USA survey of bullfrogs (*Rana catesbeiana*) and green frogs (*Rana clamitans*) in New Hampshire, USA, showed malformed frogs at 81% of the sites sampled (13 of 16 sites). Brain gonadotropin-releasing hormone, and androgen and oestradiol synthesis, hormones essential to reproductive processes, were measured in tissues taken from malformed and normal frogs. Significantly lower concentrations (nearly 3-times less) of (in-vitro produced) androgens and of brain gonadotropin releasing hormone were found in malformed compared to normal frogs. The researchers suggested that environmental factors or endocrine-disrupting chemicals that cause developmental abnormalities may also be responsible for these reduced hormone levels (Sower et al.,2000).

2.3
reptiles

Studies of long-lived species like turtles and alligators can provide a very useful indicator of the health of wetland ecosystems. In the reptile class, turtles and alligators have been the subject of numerous studies. In turtles, the following effects have been noted: abnormal production of VTG by males; deformities of the reproductive tract (including ovo-testes and shorter estimated penis length); and decreased hatching/reproduction. In alligators, the effects include: sex hormone disruption; smaller phallus; testicular abnormalities; reduced clutch viability resulting from fertilisation failure and embryo mortality; and decreased post-hatch survival.

Turtles

At a heavily polluted site on the Great Lakes in 2001, around 10% of the adult male snapping turtles (*Chelydra serpentina*) were found to be abnormally producing VTG, indicating sex hormone disruption (EC,2003).

Furthermore, studies in snapping turtles from the Great Lakes and the St Lawrence River in Canada have found differences in the physiology of adult turtles taken from highly contaminated sites compared to those from less contaminated sites. At all sites, the precloacal length of male hatchlings was larger than that of females by an equal amount at any given body size. However, the precloacal length of both males and females from the polluted site increased with body size at a slower rate than males and females from the cleaner sites. These alterations in secondary sexual characteristics are believed to be initiated early in development, are linked to contaminant levels, and may result in permanent organizational changes in morphology (de Solla et al.,2002). Precloacal length is also used as an estimator of penis length, and in a 2001 study, this was shorter in male adult turtles from the Detroit River, and in juvenile males from two polluted sites, as compared to cleaner reference sites (EC,2003)

Male snapping turtles from polluted sites in the Great Lakes are abnormally making egg yolk protein, and appear to have smaller penises.

[Photo from Nova Scotia Museum of Natural History Website]

Decreased hatching success has also been reported in snapping turtles in polluted sites around the Great Lakes compared to those from reference sites (EC,2003). At a particularly polluted site, there were no signs of reproductive activity in the adult snapping turtles (EC,2003). There is also a suspicion that deformities in Great Lakes hatchlings, which are found at higher rates than in cleaner reference locations, may be linked to chemicals.

Turtles living in polluted sites elsewhere are also affected. For example, in Lake Apopka in Florida, which is contaminated with several EDCs, many new-born red belly turtles (*Pseudemys nelsoni*) have been reported with genital disruption. Here, abnormal testes, including ovo-testes were found (Guillette et al.,1995). Also for example, male yellow-blotched map turtles (*Graptemys flavimaculata*) from a polluted Mississippi site exhibit reduced reproduction, and some males were found to have high levels of oestradiol (equivalent to levels found in females) and significantly lower testosterone (Shelby and Mendonça,2001).

Male red-belly turtles in Lake Apopka in Florida have been found with eggs in their testes. *[Photo kindly provided by Tom Coy, Austin's Turtle Page]*
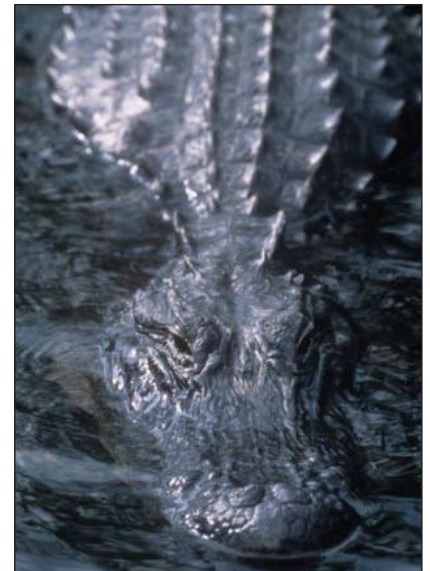
Alligators

Guillette and others have reported population decline and numerous reproductive abnormalities in alligators (*Alligator mississippiensis*) from Lake Apopka in Florida.

Professor Lou Guillette carefully examining alligator eggs. Lou's work has been pivotal in unravelling the effects of pollutants in reptiles.

This is a lake which is reported to be polluted with several organochlorine pesticides, including dicofol and DDT chemicals, following a spill in the 1980s. However, effects have also been noted in alligators from Florida lakes polluted by diffuse sources. The following reproduction-related abnormalities in Florida alligators have been suggested to be linked to exposure to EDCs: sex hormone disruption (including large adult males with higher oestrogen and lower testosterone levels than normal males of the same age); smaller phallus (penis) in males; abnormal testes; and reduced clutch viability resulting from fertilisation failure and embryo mortality (Woodward et al.,1993; Guillette et al.,1994; 1995; 2000; Guillette and Iguchi,2003).

Male alligators in polluted waters have been found with smaller phalluses and abnormal testes. *[©David Southern]*

High embryo mortality in alligators and high exposure to organochlorine pesticides has been found in Florida in Lakes Apopka and Griffin, and Emeralda Marsh, as compared to less polluted sites at Lakes Woodruff and Orange (Sepulveda,2004). Research has shown that low rates of hatching were due to fertilization failure as well as early embryonic mortality (SBRP,2003). Furthermore, recent work by Lou Guillette's team has reported increased post-hatch mortality, as well as loss of sexually dimorphic gene expression in alligators from the contaminated Lake Apopka (Milnes et al.,2008).

2.4
birds

In birds, oestrogen is the differentiating hormone for both gonads, and for behaviour (see Giesy et al.,2003). This is in contrast to sexual differentiation in mammals, where it is androgen that causes the testes to develop, such that in the absence of androgen, the female is the default sex. In birds, in the absence of oestrogen, both gonads develop into testes, whereas during normal female development, the left gonad develops into an ovary while the right gonad regresses (Fry,1995). Such differences in the control of early life developmental processes may mean that birds respond to environmental endocrine disruptors rather uniquely.

For birds to be exposed to pollutants during the critical period of development, compounds must be passed from the female bird to her eggs. DDT is known to be readily transferred to the lipid-rich yolk, but it seems that several other contaminants, including large molecules like deca brominated diphenylether (deca-BDE) can also be transferred into the egg (see EU RAR). Fish eating birds may be particularly exposed to persistent and bioaccumulating contaminants.

In birds, pollutant related effects include: abnormal VTG production in male birds; deformities of the reproductive tract; embryonic mortality; reduced reproductive success including egg-shell thinning; and poor parenting behaviour.

Male peregrine falcon in Spain have been feminised, and are abnormally making the female egg yolk protein.

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Abnormal VTG Production in Male Birds

In 2001, male herring gulls (*Larus argentatus*) from a polluted area around the Great Lakes were found with elevated levels of VTG in their blood. As in fish, this egg yolk precursor protein is normally produced by breeding females (EC,2003). Therefore, this indicates that these male birds were being feminized. A team working in Guadalajara in Spain has also found raised VTG levels in male peregrine falcon (*Falco peregrinus*), suggesting a potential ongoing threat to birds of prey. The peregrine falcon in Spain is considered vulnerable, and in this population over the last decade, a decrease in successful breeding pairs has been reported (Jiménez et al.,2007).

Deformities of the Reproductive Tract and Ovo-testes in Male Birds

There appear to be few studies of the internal reproductive tract in birds. However, in 2001, a male herring gull (*Larus argentatus*), nesting in the lower Great Lakes (downstream of a polluted area) was found with a significantly feminized reproductive tract (EC,2003).

Male herring gulls around the Great Lakes have been feminised by pollutants.

[©iStockphoto.com-Richard Thornton]

Szczys and colleagues (2001) noted that at Bird Island, off the coast of Massachusetts, the sex ratio of hatched roseate tern (*Sterna dougallii*) chicks was biased (55%) in favour of females, raising concerns about the male of the species. These observations of skewed sex ratios and female-female pairing among endangered roseate terns gave rise to investigations in common terns (*Sterna hirundo*), as a surrogate tern breeding in Massachusetts. In 1993/94, 60-90% of hatching male common tern embryos sampled exhibited ovarian cortical tissue in their testes (ovo-testes). However, examination of 21 day old common terns collected from Bird Island in 1995, suggested that the ovo-testes may become fully regressed and therefore do not lead to permanent alterations in gonadal tissue that would be expected to impair reproduction. It has also been speculated that ovo-testes might occur naturally in some common terns at hatching, although the frequency with which it occurs might be enhanced by exposure to contaminants (Hart et al.,2003).

2.4
birds
(cont)

Embryonic Mortality and Reduced Reproductive Success in Birds

Early concerns about the effects of pollutants in birds stemmed from reproductive and developmental effects that were reported in the Great Lakes, particularly in fish eating birds. One notable phenomenon was female-female pairing in herring gulls. DDE was found to cause abnormal development of male birds exposed in the egg, and therefore it was suggested this might have caused a reduction in the number of normal males returning to the breeding colony. In the early 1970s in Lake Ontario, where DDE levels were high, nests with five or more eggs (supranormal clutches) were seen as a result of two or more females occupying the same nest. Supranormal clutches were still being found 25 years later, and very few of these eggs were fertile (see EC,1997). In surveys during 2001-2004, reduced embryo viability was still seen in herring gull eggs, although the precise cause of this was not known (Fox,2005).

Numbers of bald eagles (*Haliaeetus leucocephalus*) are recovering slowly in North America. However, those nesting near the Great Lakes have greater difficulty reproducing than those nesting elsewhere, presumably because their food supply remains contaminated. Furthermore, more than half the bald eagles that do manage to hatch along the shores of the Great Lakes, die young (EC,2001).

Reduced reproduction has also been noted in eagles in the Arctic. For example, bald eagles had less offspring on Kiska Island, and this was associated with raised levels of DDE and organochlorine pesticides (AMAP,2004). Furthermore, organochlorine levels in some other species of predator birds are considered to exceed those associated with effects on reproduction (AMAP,2004; Knudsen et al.,2007).

Bald eagles around the Great Lakes and in the Arctic are not reproducing well.

[©iStockphoto.com-Frank Leung]

Tree swallows nesting along the polluted Hudson river in the USA have been found to build small, low quality nests.

[Photo kindly provided by John Peterson Myers]

There are several reports of altered parenting behaviour in birds leading to reduced reproductive success, although there is not an abundance of research in this area. For example, reproductive failure of a number of fish-eating birds was observed around the Great Lakes in the mid-1960s to mid-1970s, and investigations in the herring gull (*Larus argentatus*) showed that this was due to decreased nest attentiveness during incubation, and to direct embryotoxic effects (Peakall and Fox,1987). Similarly, in Forster's Tern (*Sterna forsteri*) at Lake Michigan lack of parental attentiveness to eggs in the nest was suggested to be associated with organochlorine contaminants, leading to reduced reproductive success, although reduced reproduction here was also related to intrinsic reduced viability of the egg (Kubiak et al,1989). The mating behaviour of birds elsewhere has been impaired. For example, altered nest building, typified by smaller nests of lower quality, were found in tree swallows (*Tachycineta bicolour*) around the PCB polluted Hudson river in the US (McCarty and Secord,1999).

More recently, pollutant-related effects on reproduction, suggested to be mediated through disruption of reproductive steroid or thyroid hormones, have been reported in glaucous gulls (*Larus hyperboreus*) breeding in the Arctic. These include altered reproductive behaviour such as lower nest-site attendance in males and reduced ability of males to maintain the temperature of the nest while incubating (Bustnes et al.,2001; 2003; Verboven et al.,2008a).

Another study of glaucous gulls in Svalbard suggested that there were contaminant-induced changes in the sex hormone levels in the eggs of glaucous gulls, and it was speculated that these could affect offspring performance over and above the toxic effects brought about by the persitent pollutants in the eggs (Verboven et al.,2008b).

Male glaucous gulls in the Arctic have disrupted parenting behaviour and the hormone levels in their eggs are abnormal.

[Photo kindly provided by US Fish and Wildlife Service]

Other experiments suggest that altered prolactin hormone levels may also be involved in the decreased reproductive success. Prolactin is an anterior pituitary hormone, closely associated with reproduction and parenting behaviours in birds. Verreault and colleagues (2008) looked at prolactin hormone levels and the concentrations of

eight persistent organohalogen contaminant classes (i.e. major organochlorines and brominated flame retardants and associated metabolic products) in the blood of wild glaucous gulls in the Arctic. They suggested that organohalogen contaminants may alter prolactin secretion in male glaucous gull and may be a contributing factor to the adverse effects observed on the reproductive behaviour, development and population size of these gulls breeding in the Norwegian Arctic.

Despite the generally lower levels of organochlorine contaminants (OCs) in Antarctic biota, some compounds may exceed the levels in equivalent Arctic species. In 65 nests of south polar skuas (*Catharacta maccormicki*), both males and females were caught, and it was found that although the concentrations of organochlorines were below those documented to have reproductive effects in other aquatic birds, the eggs of females with the higher levels of organochlorines in their blood hatched later, and their chicks were in poorer condition at hatching than those of females with lower levels. Thus, these organochlorine contaminants in female skuas may delay reproduction and reduce foetal growth. However, there were no significant relationships between organochlorines and reproductive variables in males. Nevertheless, the proportion of nests containing non-viable eggs was high (47%), although no relationship was found between the parents' residues of organochlorine contaminants measured and the occurrence of non-viable eggs (Bustnes et al.,2007). Therefore, it may be that several pollutants not measured may be a contributory factor, or that other factors are involved.

2.4
birds
(cont)

Altered sex-related characteristics and potential reduced reproduction

Male starlings (*Sternus vulgaris*) exposed experimentally to environmentally relevant levels of oestrogen mimicking chemicals develop longer and more complex songs compared to control males. In addition, these experimentally dosed males had reduced immune function.

The study also reported that females preferentially chose the more exposed males. Although this was not an effect which was measured in wildlife, but was derived experimentally, it can be deduced that inappropriate choice of mate might lead to possible population level effects, because if these males were less robust in fighting off infection, their parenting ability could be compromised (Markman et al.,2008).

Male American robins (*Turdus migratorius*) from orchards in British Columbia, exposed in the wild to elevated levels of DDT and its metabolites, had significantly altered brain development, including reduced size forebrain and song nuclei. Such reduction in the areas which are responsible for song and sexual behaviour may potentially adversely affect reproduction (Iwaniuk et al,2006).

In male American robins from orchards in Canada, the areas of the brain used for mating and song have been damaged by pollutants.

[Kindly provided by ©Albert Steen-Hansen Denmark]

Eggshell Thinning in Birds

Eggshell thinning is a notorious pollution-related effect on bird reproduction, and is caused by DDE, the degradation product of DDT (for review see Giesy et al.,2003). However, the precise mechanism of action is still not known. Effects have persisted for many years, and for example, peregrine falcons (*Falco peregrinus tundrius* and *anatum* sub-species) breeding in the Canadian Arctic, were reported to have eggshells around 10% thinner than eggs produced prior to the introduction of DDT (AMAP,2004). This is presumably due to the chemical body burden being passed on to subsequent generations, coupled with the life-time exposure of each generation. Similarly, in Greenland, thickness of the shell of peregrine falcon eggs has improved in the time period 1972-2003, but even in 2003 it was still evident to some extent (7.8%) (Falk et al.,2005). In the UK, birds of prey have not recovered from the onslaught of pesticides in some areas. For example, numbers of peregrines have not recovered in eastern Yorkshire, and over the last decade there have been declines in north Scotland, Northern Ireland and northern Wales. It is thought that persecution, pollutants, and possibly lack of food may be restricting the population (RSPB,2007).

2.5
mammals

Predator mammals in contaminated areas are at risk, because bioaccumulative contaminants can build up in the food chain. Furthermore, part of the mother's body burden of man-made chemicals is transferred to the offspring in the womb and during suckling. Even mammals in a remote area like the Arctic are under threat, because persistent organic pollutants are carried to the northern latitudes on air and ocean currents, in a process termed global re-distillation. Indeed, chemical contamination in many Arctic predator species is already at levels above those which have been reported to cause effects on reproduction in other mammals (AMAP,2004).

In mammalian species the following effects, which are discussed in more detail below, have been noted. In rodents: reduced sperm; reduced testes weight and reduced reproduction. In otters and/or mink: reduced baculum (penile bone) length; smaller testes and impaired reproduction. In seals and/or sea lions: impaired reproduction (including implantation failure, sterility, abortion, premature pupping). In cetaceans: reduced testosterone levels; impaired reproduction; and hermaphrodite organs. In polar bears: intersex features and deformed genitals; reduced testes and baculum length; reduced testosterone levels in adult males; and reduced cub survival. In black bears: undescended testes. In the Florida panther: undescended testes; altered hormone levels; abnormal sperm and low sperm density. In deer: antler deformities; undescended testes; and testicular abnormalities, including cells predictive of testicular cancer. In eland (an antelope): abnormal testes, including impaired spermatogenesis.

It has been estimated that almost 1 in 4 mammalian species are at risk of extinction (IUCN,2008a). Many factors are to blame, particularly including habitat degradation, but nevertheless this highlights the need to protect mammalian reproductive capability.

First born calves of Florida bottlenose dolphins get most pollutants from their mothers and have lower survival rates than subsequent offspring.

[©iStockphoto.com-Derek Burke]

2.5
mammals
(cont)

Feral Rodents

Studies on rodents living in highly contaminated areas show effects on reproduction and the testes. For example, significantly reduced testes weights have been reported in male white footed mice (*Peromyscus leucopus*) inhabiting PCB and cadmium contaminated land. Effects on reproduction were also noted, with numbers of juveniles and sub-adults reduced compared to an unexposed population (Batty et al.,1990). Similarly, a study of striped mice (*Rhabdomys pumilio*) from a contaminated South African nature reserve reported two male animals without any sperm, and other animals with relatively low mean cauda epididymal sperm count (Bornman et al.,2007). Other rodent species reported to be affected by pollutants, include meadow voles (*Microtus pennsylvanicus*) from the infamous Love Canal waste site at Niagara Falls. Reduced population density and reduced seminal vesicle weight were reported in male animals from this polluted site compared to animals from a cleaner site (Rowley et al.,1983).

Male striped mice from a polluted nature reserve in South Africa have been found with no sperm.

[©iStockphoto.com-Nico Smit]

Otters

The Eurasian otter (*Lutra Lutra*) is the most widely distributed of all 13 species of otters, and is found in the UK and elsewhere. Several decades ago, otters *(Lutra lutra)* completely disappeared in some UK and European rivers, due to contaminant induced reproductive problems (Mason and Macdonald,2004). Monitoring in 1989-1991 suggested that at least in some areas, PCBs were still sufficiently high to exert detrimental effects on some UK otters (Mason and Macdonald,1994). In addition to the deleterious effects of PCBs and other organochlorine contaminants, habitat destruction has also had a negative impact (see EA, 2003).

After populations of otters plummeted in Europe, captive bred otters were released in some river catchments in the UK, and elsewhere (Fernandez-Moran et al.,2002), and otters are now breeding again. In some UK rivers the population growth has been slow (Mason and Macdonald, 2004), although otter populations are now expanding over much of Europe. Nevertheless, the Eurasian otter is still recognised by IUCN (International Union of Nature Conservation) as 'near threatened' (IUCN,2008b). In some European countries, such as Denmark, the distribution range of the otter was reported to be still much reduced (Pertoldi et al.,2001). Similarly, in southern Sweden, total PCB concentrations are still high and the indications of population improvement are weak (Roos et al.,2001). Overall, in the EU, the otter population distribution is still reduced, and as well as PCBs and

other organochlorine contaminants, rodenticides are also a concern in some areas (Fournier-Chambrillon et al.,2004).
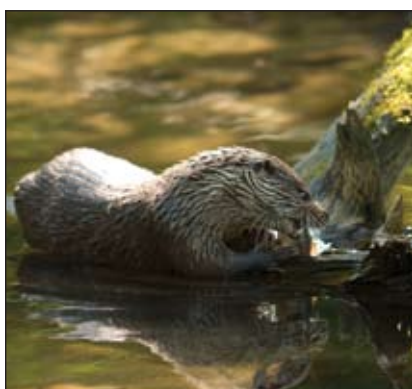
Otters in Europe have suffered reduced repro-
duction, and males with undescended testes
have been found in the UK.

[Kindly provided by ©Hugh Jansman, Alterra]

Sea otters have also declined in some areas, including the southern sea otter (*Enhydra lutris nereis*) population in California and the Alaskan sea otters (E. lutris kenyoni) in the Aleutian Islands, USA, but the reasons for this are unknown (Hanni,2003).

The North American river otter (*Lontra canadensis*) also presently occupies a greatly reduced range, and at least 17 states and one Canadian province have undertaken re-introduction programmes (Kimber and Kollias, 2000). Chemical pollutants have been suggested as a possible cause of the decline in both Europe and North America (Conroy et al.,2000; Wren,1991). Structural defects of the male reproductive tract have been reported in some studies of male otters. Otters surveyed in the polluted Lower Columbia river in North America in the 1990s were reported to have

abnormally small reproductive organs, and these reproductive tract disorders correlated with several environmental contaminants present in the river (NBS,1996). Research by Henny and colleagues reported that the baculums and testicles of young males from the Lower Columbia River were shorter or smaller than in animals of the same age group from non-polluted areas. In the Portland Vancouver area, where the highest PCB and organochlorine levels were recorded, of the four animals collected, one otter even had no testicles. However, it was suggested that some of the effects on the young male river otters from the Lower Columbia River might be temporary, resulting from delayed development due to endocrine dysfunction (NBS,1996). In addition, it may be that PCBs are not responsible for the effects on the baculum of the otter, but are just a 'tracer' for other pollutants, because an experiment in which growing mink were fed Arochlor 1254 PCB did not report any effect on their baculi (Aulerich et al.,2000).

The range of North American river otters has
reduced. In a polluted river, males with smaller
baculums and testicles have been reported.

[Kindly provided by ©Nicole Duplaix]

In the UK, the Environment Agency funds post mortem examination of otters found dead, mainly due to road kill, in England and Wales. One unilateral cryptorchid male otter was found in 1994, and although no further males with undescended testes were reported in the 600 or so found dead in south west England (Simpson,2008), a study of male otters from southern England showed smaller baculum length in young otters was correlated to higher levels of organochlorine contaminants in their livers. Out of the 195 males examined, abnormally small or distorted baculi were seen in 7 otters, with the otter from Hampshire having both a small penis and unusually small testes (Simpson,2007). However, these results need careful interpretation. In depth examination of the testes from more than 250 or so male otters found dead, which have been frozen and stored in a 'bio-bank' serving much of England and Wales, could provide more information as to whether the reproductive health of male otters in the UK was compromised. However, in 2008 in the UK more otters with undescended testes have been found than in previous years (Chadwick,2008). Early in 2008, one otter from Humberside was found with both testes undescended, and subsequently two other otters with unilateral cryptorchidism were found in the summer of 2008, one in Cumbria, and one in Hertfordshire (Chadwick,2008). More funding is needed for further detailed investigation of the bio-banked specimens, and or for in-depth investigation of fresh new specimens.

2.5
mammals
(cont)

Mink

The endangered European mink (*Mustela lutreola*) has suffered a rapid decline, and its distribution is still shrinking. In France, the range of the mink shrank by nearly 50% over the last 20 years (Fournier-Chambrillon et al.,2004). Rodenticide exposure via prey (Fournier-Chambrillon et al., 2004), and exposure to contaminants such as PCBs and other organochlorines are a concern. It seems that mink are particularly susceptible to reproductive effects due to dioxins and structurally related PCBs (Wren,1991), and to mercury (Basu and Head,2008).

Mink in Europe have suffered a rapid population decline.

[©iStockphoto.com-Frank Leung]

In North America, in the 1970s, commercial mink farms reported reproductive failure in their mink which were fed fish from the Great Lakes, and it was subsequently shown that exposure to low levels of PCBs could impair reproduction in this species. Data from 1982 and 1987 from surveys around the Great Lakes continued to indicate that wild mink (*Mustela vison*) populations were being affected by pollutants, particularly PCBs (Wren,1991). Rather alarmingly, recently reported levels of PCBs in mink from western Lake Erie have increased since 1979 when they were last sampled, and moreover, many exceed the lowest observable effect level for reproductive impacts (EC,2003; Fox,2005).

The Housatonic River in Connecticut, downstream of an old General Electric Company plant, is particularly contaminated with PCBs. Mink fed fish from this river had offspring with lower birth weights and higher infant mortality rates, compared to mink fed with Atlantic herring, such that it can reasonably be predicted that the wild population are likely to be suffering adverse effects (Bursian et al.,2003). Structural defects have also been noted. In British Columbia in Canada, there was a significant negative correlation between total PCB concentrations and baculum length in juvenile mink, caught in the winters of 1994/5 and 1995/6, although individual animals with gross abnormalities of reproductive systems did not show high levels of chlorinated contaminants (Harding et al.,1999). Moreover, as reported in the section above on otters, it may be that PCBs are not the causal agent for this structural defect, but instead a marker for other contaminants,

because an experiment in which growing mink were fed Aroclor 1254 PCB did not report any effect on their baculum (Aulerich et al.,2000).

Seals and Sea Lions

In the 1970s, harbour seals (*Phoca vitulina*) in the polluted Dutch Wadden Sea (part of the North Sea) declined in number, with low reproduction being blamed on PCBs adversely affecting female reproductive capability. A study showed that female harbour seals fed fish from the polluted Wadden Sea had half as many pups compared to seals fed fish from the less contaminated Atlantic (Reijnders,1986). Altered levels of sex hormones were suggested to have led to implantation failure (Reijnders,1990).

Subsequently, seal populations in Britain and Europe were decimated by outbreaks of phocine distemper virus in 1988 and 2002. These outbreaks caused the deaths of more than 23,000 and 30,000 harbour seals respectively and the initial outbreak was suggested to have been exacerbated by pollutants compromising the immune system of the seals (Härkönen et al.,2006; Hall et al.,1992). Concern about this fish eating mammal is still high. Britain holds 40% of the total European common or harbour seal (*Phoca vitulina*) population, and the numbers of harbour seals in eastern England have not increased since the end of the 2002 phocine distemper epidemic. Indeed, there is evidence of a general decline in large harbour seal colonies around Britain, apart from the Inner Hebrides where numbers are stable

or increasing. The role that pollution may play in this is under investigation, but whatever the causes it seems that they cover a large part of the North Sea as there has been widespread population declines ranging from the Wash to the Shetland (Lonergan et al.,2007). The Sea Mammal Research Unit at St Andrew's university is going to look at some parameters related to the reproductive health of male and female seals captured and released in August and September 2008 at a number of sites. Scottish Natural Heritage is funding some research to enable the seals found dead to be subject to investigation, but more funding is needed to fully investigate the potential effects of contaminants on the reproductive health this species (Hall, 2008).

Common seal colonies around Britain have declined.

[©iStockphoto.com-Janne Hämäläinen]

In the Baltic, it is well accepted that pollution has caused reduced numbers of seals due to reproductive problems. The seals are reported to have compromised endocrine systems associated with high levels of PCB and DDT and related chemicals

(see review, Damstra et al., 2002). Some of the disorders observed in the exposed female seals included abortion in early pregnancy, uterine stenosis and occlusions, and sterility (Bergman,1999a; Bergman 1999b).

Moreover, many seals from the Arctic, including some ringed (*Phoca hispida*) and northern fur seals (*Callorhinus ursinus*) are contaminated with summed PCB levels above the threshold for decreased reproduction in otter (AMAP,2004).

In sea lions (*Zalophus californianus*) in the USA stillbirths and premature pupping were reported in the 1970s, and this was associated with high PCB and DDE levels (DeLong et al.,1973). At this time, on San Miguel Island some twenty percent of the California sea lion pups died due to premature birth. The p,p'-DDE levels in the premature parturient cows' blubber were 7.6 times greater than in the full-term animals, although it seems that infections may also have contributed (Gilmartin et al.,1976). In western Alaska, Steller sea lion (*Eumetopias jubatus*) populations have also suffered a decline. The cause is not known, and it may be related to a decline in their prey, but pollution may also be a factor, because these sea lions have been found to have higher concentrations of persistent organic pollutants in their excreta, than less affected populations (AMAP,2004).

2.5
mammals
(cont)

Cetaceans (whales, dolphins and porpoise)

Beluga whales (*Delphinapterus leucas*) in the St Lawrence estuary and orca whales (*Orcinus orca*) in the Northeast Pacific are two very highly polluted wildlife populations (for review see Fossi and Marsili,2003; Heiman et al.,2000; McKinney et al.,2006; Wolkers et al.,2007). Neither is reproducing well. They have some of the highest PCB levels found in wildlife, higher than those associated with reduced reproduction in seals, although the effects of contaminants on whales is difficult to ascertain with certainty (Trites and Barrett-Lennard,2001).

The beluga population in the St Lawrence estuary has not increased since hunting was banned in the 1970s. In a report published in 1995 by Douglas, the rate of pregnancy of beluga whales in the St Lawrence river estuary was only 3% compared with 35% in those from the Canadian Arctic (see Riedel et al.,1997). De Guise (1995) suggested that this could be linked with exposure to organochlorines. Scientists have also reported finding a true hermaphrodite (with 2 testicles and 2 ovaries) (De Guise,1995), and a pseudo-hermaphrodite (Mikaelian et al.,2003).

Beluga whales in the St Lawrence estuary are highly polluted. Reproduction is imparied and hermaphrodites have been found.

[©iStockphoto.com-Klaas Lingbeek- van Kranen]

In the Arctic, not only killer whales, but also some harbour porpoises *(Phocoena phocoena)* from Norway, long-finned pilot whales (*Globicephala melas*), narwhal (Monodon monoceros), and some minke whales (*Balaenoptera acutorostrata*) have summed PCB levels higher than those associated with decreased otter and mink reproduction (AMAP,2004).

There has been a decline in harbour porpoise (*Phocoena phocoena*) populations since the 1940s in the southern North Sea and the English Channel, and similar declines have been reported in the rest of Europe. Recent studies mainly suggest that the pregnancy rate in North Sea porpoise is lower than in the western Atlantic or Iceland waters (Pierce et al,2008). At least some of this population decline is believed to be due to chemical contamination of the female. For example, a study of stranded animals found concentrations of PCBs in the fat of female common dolphins (*Delphinus delphis*) and harbour porpoises from the Atlantic coast of Europe above the threshold at which effects on reproduction could be expected in other mammals (such as mink, otter and seals) in 40% of the dolphins and 47% of the porpoises. In the southern North Sea, this rose to 74% of porpoises being over the contamination threshold at which reproduction effects could be expected in other mammals. Perhaps not surprisingly therefore, the average pregnancy rate recorded in porpoises in the southern North Sea study area was lower than in the western Atlantic, although the scientists cautioned that the sample size for the southern North Sea was small (Pierce et al.,2008).

Porpoise numbers have declined. Some stranded animals are contaminated to levels likely to affect reproduction.

[Photo kindly provided by Paul Jepson,©MEM]

The reproduction of dolphins in waters off the USA also seems compromised. For example, in Sasasota Bay, Florida, female reproductive success improved with age, potentially reflecting depuration of the mother's pollutants to her first born, and correlating with the mother's PCB load. Only half of the first born calves of bottlenose dolphins (*Tursiops truncates*) survived through their first year, compared to a 70% survival rate in subsequent calves (Wells et al.,2005).

Effects on sex hormones in cetaceans have been associated with pollutants. For example, reduction in the testosterone levels in Dall's porpoise of the northwestern North Pacific has been associated with exposure to PCBs and DDE, the latter being statistically significant (Subramanian et al.,1987). The authors considered that, "irrespective of the precise mechanisms involved, the plausible effect of PCBs and DDE in reducing the testosterone level in Dall's porpoise may be an indication of the causative effects of PCBs and DDE in affecting the normal sexual functions of wild animals."

Without good base-line data from unpolluted populations it is difficult to know whether organ size has been impacted by chemical exposures, but a study of 192 male porpoises found stranded or caught in commercial fishing nets in UK waters, found no consistent evidence that any of the contaminants in the porpoises were negatively associated with male reproductive indices (Bennet et al.,2003).

2.5
mammals
(cont)

Polar Bears

Some PCBs have increased in polar bears (*Ursus maritimus*) in the period between 1967 and 1994 (Derocher et al.,2003) and similarly, other "newer" contaminants have also increased in recent years (1984-2006) (Dietz et al.,2008). Indeed, it is considered that pollutants coupled with global warming may pose a threat to the long-term survival of this species (Jenssen,2006).

The impacts of contaminants on the Svalbard polar bear population are inconclusive but there are suggestions of contaminant-related population level effects that could have resulted from reproductive impairment of females, lower survival rates of cubs, or increased mortality of reproductive females (Derocher et al.,2003). Cubs of mothers with high levels of contaminants in their fat were found to be more likely to die during their first year than cubs of mothers with low levels. These cubs may be particularly vulnerable since polar bear milk is about 30 percent fat, and contaminants stored in the mother's fat are transferred to offspring during suckling. Certainly, the summed PCB levels in some Svalbard polar bears exceeded the levels known to be correlated with poor reproductive success in seals (AMAP,2004).

Male polar bears in East Greenland with higher levels of organochlorine pollutants were found to have smaller testes and shorter baculums.

[©iStockphoto.com-David T.Gomez]

Intersex / Hermaphroditism and Reduced Testosterone in Polar Bears

Scientists have suggested that in polluted areas, hermaphrodite polar bears may be more common, and that this condition could be due to excessive maternal androgen excretion caused by a tumour or endocrine disrupting pollutants (Wiig et al., 1998). In 1996, two yearling Svalbard polar bears, which were believed to be female, were found with a normal vaginal opening and a 20 mm penis containing a baculum. Then on subsequent separate occasions, two other Svalbard bears were found to exhibit female pseudo-hermaphroditism as they had deformed genitals, manifest as clitoral hypertrophy (Wiig et al.,1998). However, some of these later reported females may have been mis-diagnosed as pseudo-hermaphrodites, because a subsequent female was found with an enlarged clitoris, with no signs of any histological or structural changes which would be expected if hormone disruption was involved (Sonne et al., 2005). Nevertheless, subsequent studies on 44 female East Greenland polar bears which had been caught by subsistence hunters, indicated that contaminants were affecting oestrogen-sensitive female organs. For example, an inverse relationship was found between the levels of the sum of some organohalogen contaminants and ovary length and ovary weight (Sonne et al.,2006)

Normal sexual development and later reproductive function are dependent on testosterone, and so it is a matter of some concern that the levels of some organochlorine contaminants in adult male polar bears have been linked with perturbation of testosterone levels. Researchers have suggested that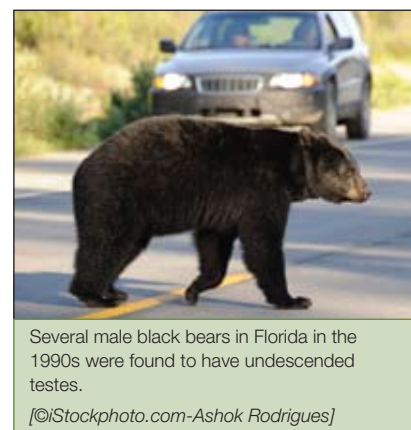 the continuous presence of high concentrations of organochlorines in male polar bears throughout their life could therefore aggravate any reproductive toxicity that might have occurred during early development in the womb (Oskam et al.,2003).

The genitalia in male polar bears also appear to be damaged by pollutants. Reproductive organs from 55 male East Greenland polar bears caught by subsistence hunters were examined to investigate the potential negative impact caused by organohalogen pollutants. There was a significant inverse relationship between organohalogen contaminants and testis length and baculum length and weight. This was found in both sub-adults (associated with DDT related chemicals, dieldrin, chlordanes, hexacyclohexanes, PCBs, and polybrominated diphenyl ethers (PBDEs)) and adults (hexachlorobenzene (HCB)). Baculum bone mineral densities decreased with increasing chlordanes, DDTs, and HCB in sub-adults and adults. It seems that hormone disrupting pollutants are reducing the size of male East Greenland polar bear genitalia, and this may pose a risk to this polar bear population in the future because of reduced sperm and penis size/robustness (Sonne et al.,2006).

Black Bears

Effects suggestive of sex hormone disruption have been reported. For example, Dunbar and colleagues found retained testes in 11 (16%) of 71 male black bears (*Ursus americanus*) examined over a 3-year period in Florida (USA). Four of the 11 bears were over one year old and were therefore considered to be cryptorchid. The remaining seven bears may have had delayed testicular descent due to immature development. This is the first published report of the prevalence of cryptorchidism in black bears, and therefore it is difficult to draw conclusions (Dunbar et al.,1996). As of 2008, there did not seem to be any significant reproductive problems in Florida black bears (Cunningham,2008).

Several male black bears in Florida in the 1990s were found to have undescended testes.

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In bears in Alberta in Canada, a couple of decades ago there were some reported cases of masculinized females, but the cause of this pseudo-hermaphroditism was not known (Cattet, 1988). Out of 38 black and 4 brown bears collected, 4 female black bears and 1 female brown was found to have some degree of male genital development. Given the frequency of the occurrence, environmental factors might have played a role. It was suggested that excessive maternal androgens, herbicides, or plant derived alkaloids might have been involved. However, it is speculated here that atmospherically transported industrial pollutants could perhaps also have played a role.

2.5
mammals
(cont)

Florida panthers have been found with abnormal hormone levels, reduced sperm and undescended testes.

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Florida Panther

Facemire and colleagues (1995) reported that many of the small endangered population of Florida panther (*Felis concolor coryi*) have abnormal sperm, low sperm density, or undescended testicles. These effects may be linked to the abnormally similar serum oestradiol levels found in male and females, which indicated that many males had been de-masculinized and feminized. Endocrine disrupting pollutants, taken up through the food chain, were considered to be the possible cause. However, other researchers have suggested that the effects seen may be largely due to the genetics of the in-bred small population (Mansfield and Land,2002). Both sterile males and an infertile female have been reported (Facemire et al.,1995). High levels of mercury, PCBs and pesticide residues have been reported in Florida panther (see Florida Fish and Wildlife Conservation Commission).

Deer – Family Odocoileus

There are several examples of male reproductive health problems in deer. For example, Veeramachaneni and colleagues have reported that many Sitka black tail deer (*Odocoileus hemionus sitkensis*) on Kodiak Island, Alaska have undescended testicles, and antler dysgenesis, both of which are signs of defective androgen action. These researchers could not rule out a link with a recessive mutation in a founder animal, but considered that in-utero exposure to one or more environmental oestrogen mimicking chemical was more likely to be the cause.

In the Aliulik peninsula on Kodiak Island, two thirds of the deer examined (61 out of 94) had neither testicle descended, and 70% of these (43) had abnormal antlers. Another 7% had one testicle un-descended. Animals from elsewhere on the island or from neighbouring islands were much less affected. The testes of some of the deer were subject to examination. Where both testes were un-descended, there were many abnormalities, including no spermatogenesis and carcinoma in situ (CIS) like cells (considered to be precursors of seminoma, a form of testicular cancer) (Veeramachaneni et al.,2005). Effects on reproduction at the level of the individual must therefore be apparent, as those with neither testicle descended are azoospermic (without sperm).

Many male Sitka black-tailed deer on Kodiak Island have undescended testes and de-formed antlers.

[Photo kindly provided by Dr.James.P.Jacobson]

From 1996 to 2000, accident-killed and injured white-tailed deer (*Odocoileus virginianus*) in Montana USA were collected and examined for genital abnormalities. Of the 254 male deer examined, approximately two thirds showed varying degrees of genital developmental anomalies, specifically mis-positioned and undersized scrota and ectopic testes. The authors discussed the possible role of endocrine disrupting pesticides, but could not give any firm conclusions as to the cause of the abnormalities (Hoy et al., 2002).

Earlier research by Marburger (1967) also reported finding genital defects in male white tailed deer (see Veeramachaneni et al., 2005). Similarly, earlier research in Columbian black tailed deer in California (*Odocoileus hemionus columbianus*) found effects suggestive of defective androgenic action. During an 18 year period, 4.1% of male deer had mis-shapen antlers and some had testicular atrophy, but the cause was not determined (De Martini and Connolly,1975).

Mule deer (*Odocoileus hemionus*) on a former plutonium production site along the Columbia River at the Hanford Site, Washington (USA) have also been found with abnormal testes and antlers. Some 27 of 116 adult males examined had unusually shaped, velvet-covered antlers and abnormal testes. The severity of the testicular atrophy and apparent lack of other affected tissues led the researchers to suggest that radiation was unlikely to be responsible (Tiller et al.,1997).

Eland

The eland is a large antelope from the Bovidae family. Deformities reported in eland (*Tragelaphus oryx*) are believed to be the first evidence of terrestrial wildlife being affected by hormone disrupting chemicals in South Africa. Professor Bornman and colleagues collected testes and body fat from 24 eland. Focal white gritty areas were observed in the testes of all eland, and spermatogenesis was generally impaired (Bornman et al.,2007). The fat samples of the eland contained the oestrogenic compound p-nonylphenol, and it was suggested that the testicular lesions observed in eland could be associated with this, as the vacuolisation of sertoli cells were similar to those observed in rats exposed to nonylphenol (Bornman et al., 2007). Nonylphenol ethoxylate, which breaks down to nonylphenol, is used as an ingredient in many pesticides, as well as in leather or textile processing, metal working, and cleaning

Eland in a polluted South African nature reserve have been found with abnormal testes and impaired spermatogenesis.

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operations. In some of the eland, other endocrine disrupting pollutants, including octylphenol, PCB and DDT related chemicals were also found. A few atypical germ cells were seen, but detailed morphological evaluation to show carcinoma in situ was not possible because of limitations of tissue fixation and processing (Bornman et al.,2007) These workers concluded that the findings in eland were similar to the testicular dysgenesis syndrome in humans attributed to developmental exposures to chemicals.

section 3
effects reported in wildlife in polluted environments, endocrine disruptors in the environment and the mixture effect

3.1
highly contaminated aquatic environments

In the Great Lakes basin over 400 man-made chemicals have been found in wildlife, and reproduction has been impaired in at least 14 species.

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Persistent chemicals tend to end up in water-bodies and oceans, where elevated levels of bioaccumulating compounds are found in wildlife at the top of the food web. Most of the data on the adverse effects of chemicals in wildlife come from polluted waters that have been the subject of in-depth studies. These particularly include the Great Lakes, the Baltic and the Arctic, the latter being particularly contaminated due to long range transport of pollutants via air and ocean currents, in a process called global re-distillation.

In the Great lakes basin, over 400 man-made chemicals have been found as wildlife contaminants (EC,1997). By the early 90s, at least 14 species of fish and fish-eating wildlife had been reported with reproductive problems, population declines or other adverse health effects attributed to chemical contaminants (Gilman et al.,1991). These species included snapping turtle (*Chelydra serpentine*), cormorant (*Phalacrocorax auritus*) black crowned night heron (*Nycticorax nycticorax*), bald eagle (*Haliaeetus leucocephalus*), osprey (*Pandion haliaetus*), herring gull (*Larus argentatus*), ring backed gull (*Larus delawarensis*), Caspian tern (*Hydroprogne caspia*), common tern (*Sterna hirundo*), Forster's tern (*Sterna forsteri*), wild mink (*Mustela vison*), otter (*Lutra canadensis*), beluga whales (*Delphinapterus leucas*) from the St Lawrence river which is fed by water from the Great Lakes, and farmed mink fed Great Lakes fish. Levels of some contaminants have declined since the early 1990s, but concern is still high. Therefore, in 2001, Environment Canada (EC) initiated the first phase (2001-2005) of the Fish and Wildlife Health Effects and Exposure Study, which was to explore the wildlife / human health connection. Some fish are still contaminated to an extent such that it is recommended they should only be eaten in limited amounts or not at all.

The Baltic has also been the subject of numerous studies as it is relatively highly contaminated due to its shallowness and low water exchange. The Finnish Food Safety Authority has recommended that women of childbearing age should limit their consumption of Baltic herring, salmon and predatory fish (see EVIRA,2008). Effects have been found in fish eating wildlife, particularly seals (Bergman

and Olsson 1985; Bergman 1999a). It would appear that many female seals have been unable to reproduce because of persistent pollutants (Bergman,1999), and although the Grey seal (*Halichoerus grypus*) population has increased following a decrease in some organochlorine levels, problems remain (Nymen et al.,2002) In many marine mammals in both the Baltic and the North sea, effects on the immune system is an ongoing concern, because there is evidence to suggest that mass mortalities may have arisen due to a reduced ability to fight off infections (van Loveren,2000; Hall et al.,1992; Jepson et al.,2005).

Helsinki harbour. Seals in the Baltic have had problems reproducing, and young women are advised to limit their consumption of Baltic fish.

[©iStockphoto.com-Dan Mason]

In the Arctic, levels of contaminants are considered to be above the threshold for effects in a few wildlife species (Fisk et al.,2005). Effects on immune system function associated with contaminants have been reported in polar bears and humans (for reviews see, Muir et al.,2005; van Oostdam et al.,2005). Indeed, for other adverse effects safety margins may not be sufficient to provide an adequate level of protection for the human population. For example, researchers exposed rats to a mixture of contaminants that simulates the blood contaminant profile of humans residing in the Canadian Arctic. Female rats were exposed during pregnancy and lactation to this mixture at doses that resulted in

blood levels that were just 100-fold higher than the blood level found in humans living in the Canadian Arctic. Many adverse effects were noted in the offspring they produced including growth suppression, decreased spleen and thymic weights, increased serum cholesterol and liver microsomal enzyme activities, lower liver retinoid levels, and histological changes in the liver, thyroid, and spleen (Chu et al.,2008). Similarly, experiments feeding farmed Arctic foxes (*Vulpus lagopus*) with whale blubber 'naturally' contaminated in the wild, has illustrated that wild foxes exposed to an environmental cocktail of persistent organic pollutants may be at risk of developing chronic kidney and liver damage (Sonne et al.,2008).

Arctic foxes are at risk, because captive foxes fed on whale blubber show many adverse health effects.

[Kindly provided by Per Michelsen © www.arcticimages.com]

In the last decade, research funding has led to increased knowledge about the effects of chemicals in the Arctic (Fisk et al.,2005), but there are concerns in some quarters that funding for wildlife studies appear to be now being cut back. The global UNEP Stockholm Convention which came into force in 2004 has resulted in the phase out, or reduced releases, of some 12 organochlorine chemicals, which in turn has reduced some levels recorded in the environment (Chan et al.,2008). However, levels of other persistent contaminants have increased in recent years (Dietz et al.,2008).

3.2
endocrine disruptors and mixture effects

Some unidentified chemicals in the discharges of most sewage works tested are able to block the action of male hormones.

[Photo kindly provided by ©Anglian Water]

Information from laboratory studies can also be used to assess potential effects. For example, in the UK it is a matter of some concern that two nationwide surveys in 2003, which assessed the effluent from 43 sewage treatment works on 2 separate occasions using the YAS (yeast androgen assay), have revealed that 77% of samples had anti-androgenic activity of 50 micrograms per litre (μg) flutamide equivalent (FE) or more, 17% had between 300-500 μg/l FE, and 7% had 500 μg/l FE or more (Johnson et al.,2007).

Flutamide is an anti-androgenic pharmaceutical compound which has been used as a comparative measure of anti-androgenic activity. Anti-androgenic pollutants have the potential to de-masculinise males and affect their ability to reproduce. As of November 2008, the chemicals responsible for the anti-androgenic activity in the effluents had not yet been identified. In addition to contaminants with anti-androgenic effects, many effluents also contain chemicals with oestrogenic action

and these contaminants have the ability to feminise males. In UK sewage effluents, natural oestrogens, including oestrone and17β-oestradiol have been found, alongside pharmaceutical oestrogens, such as ethinyl oestradiol, used in the birth control pill, and these compounds typically account for the bulk of the oestrogenic activity in these discharges. However, several man-made chemicals with oestrogenic action have been found in fresh water river sediment, including bisphenol A, octylphenol and nonylphenol (Pottinger et al.,2008). With regard to the marine environment, man-made endocrine disruptors have been reported in estuaries and discharges, including, for example, nonylphenol and DEHP (di-(2-ethylhexyl) phthalate). Higher oestrogenic activity has been found in estuarine sediments, as compared to estuarine water, and nonylphenol, cinnarizine (an anti-histamine drug) and cholesa-4,6-dien-3-one (a natural cholesterol degradation product) were positively identified as making a minor contribution to this activity. Nevertheless, the main cause of the oestrogenic activity in estuarine sediments is unknown (Allen et al., 2002). There is therefore a need for more resources to identify the pollutants found in the environment that are responsible for endocrine disrupting activity.

In laboratory experiments, many more chemicals have been shown to have sex hormone disrupting properties or to affect androgen dependent tissues, and some of these substances have also been found in the environment (EA,1998). For example, some polybrominated diphenyl ethers (PBDEs) (Stoker et al.,2005; Darnerud,2008) which have had extensive use as flame retardants in consumer products, and some PCBs and dioxins are frequently found as contaminants in biota (Gray et al.,1999a; Kaya et al.,2002; see Vreugdenhil et al.,2002) . Moreover, certain phthalates (Howdeshall et al.,2008) and parabens (Darbre and Harvey,2008) have been reported to possess anti-androgen activity in animals, as have many pesticides. These include the DDT metabolite, p,p'-DDE, linuron, vinclozolin, procymidone, prochloraz (Rider et al.,2008; Wilson et al.,2008), fenarimol (Ankley et al.,2005), fenitrothion (Katsiadaki et al.,2006), chlorpyrifos (Kang et al.,2004), and ketoconazole (Shin et al.,2006). Dieldrin, a persistent pesticide, is also reported to have anti-androgenic properties in test tube experiments (Fowler et al.,2007). In addition, certain pharmaceuticals have de-masculinizing properties, some of which, such as the drug cimetidine used for indigestion and ulcers, may leave the body unchanged in urine or breast milk (Huques et al.,2000; França, et al.,2000; Hey,2006)

Epidemiological and field studies which seek to identify the chemicals that are instrumental in causing adverse effects in the wild are hampered by the fact that there are many confounding factors, and pollutants are ubiquitous. Under natural conditions, it is virtually impossible to identify individual causative agents in mixtures of highly correlated contaminants. Nevertheless, associations can be seen between effects and certain contaminants.

Controlled laboratory experiments continue to play a major part in determination of the potential causal agent(s). Unfortunately, however, laboratory studies are severely hampered because such a 'reductionist' approach cannot embrace the complex multi-causality and stepwise triggers that may impact on disease in the outside world. For example, in the environment the presence of several pollutants, natural ecosystem stressors, and other factors, such as ultra violet light exposure (Lyons et al.,2002), may affect the toxic response.

[1] Dioxins are chlorinated chemicals which are products of incomplete combustion.
[2] Phthalates are used in many consumer products and are found as uniquitous environmental contaminants. Amongst other things, they are used to make plastics flexible.
[3] Esters of p-hydroxybenzoic acid (parabens) have been found in human urine and in human breast tissue. Parabens is used in many bodycare cosmetics, and is able to penetrate the skin (Darbre and Harvey,2008)

3.2
endocrine disruptors and mixture effects
(cont)

Wildlife and humans are exposed to many pollutants. This mixture or so-called 'cocktail effect' can act additively and tip susceptible animals over the threshold for effects.

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Mixture Effects

Wildlife are exposed to many compounds concurrently, some of which act with a similar mechanism of action or via mechanisms of action that converge. For example, laboratory experiments have now clearly shown that additive effects can occur due to exposure to more than one chemical. For example, two phthalates with a similar mechanism of action, working against the male hormone, can give rise to additive effects (Foster et al.,2000). Similarly, subsequent experiments have revealed that in rodents, five phthalates show additive action, with BBP (benzylbutyl phthalate), DBP (di(n)butyl phthalate), DEHP (di(2-ethylhexyl) phthalate) and DiBP (diisobutyl phthalate) being equipotent, and DPP (dipentyl phthalate) being about 3-fold more potent (Howdeshell et al.,2008). Furthermore, toxicants can produce additive effects even when two or more chemicals are acting via different mechanistic pathways (Gray et al.,2002; Rider et al.,2008). For example, certain phthalates inhibit testosterone synthesis during foetal life, and dioxins alter androgen dependent tissues, and also the pesticides vinclozolin, procymidone, linuron and the DDT metabolite (p,p'DDE) are all androgen receptor antagonists, which de-rail the process of masculinization (Gray et al.,2001; Wilson et al.,2008). Male rodents exposed experimentally to such anti-androgenic chemicals show a cascade of features representative of malformations in male hormone receptor dependent tissues, including shortened ano-genital distance, nipple retention, and testes-related effects (Gray et al.,1999a). Hass's team have also found similar additive effects in rodents after exposing them simultaneously to three anti-androgen mimicking chemicals. An increased number of penile defects (hypospadias) was found after exposure to the mixture, but no such increase over normal levels was found when each chemical was administered alone at the same concentration as in the mixture, because individually it was below its effect level (Christiansen et al.,2008)

Interactive effects, including additivity or even synergism, also occur with other mechanisms of action. For example, additivity of effects has been shown in fish exposed to a mixture of oestrogenic chemicals (Brian et al.,2005), and similarly Crofton and colleagues (2005) have shown that when several thyroid disrupting chemicals are given to rodents, effects can occur even when each chemical is given at a dose level below their

no effect concentration. For thyroid disrupting chemicals, both additive and synergistic effects were reported.

Several workers have shown that exposure to pesticide mixtures can cause effects greater than single exposures. For example, Moore and Lower (2001) have shown an additive response in male fish due to exposure to a weak mixture of atrazine and simazine pesticides (0.5ppb each), which caused reduced milt expression in fish because of altered olfactory function and reduced reaction to female pheromones. Similarly, Hayes and co-workers have shown effects on the immune system in frogs exposed to low levels of several pesticides (Hayes et al.,2006).

Organisms are most vulnerable to the effects of EDCs during early life development in the uterus or in the egg, and during puberty, particularly when cell fate is determined and differentiation is occurring. Moreover, there may be a substantial delay before the effects from early life exposures are seen. Effects from exposures prior to birth or hatching can range from gross structural defects to subtle, but important, behavioural effects. This delay between exposure and effects further hampers the identification of which chemicals are involved.

3.3
transgenerational effects

Effects due to early life exposures may also result in effects on subsequent generations. Several laboratory studies have suggested transgenerational effects in both invertebrates and vertebrates. A transgenerational effect is an effect which is carried across generations as a consequence of events that happened during the lifetime of the previous generation. Such effects have been seen in fish (Gray et al.,1999b), and also in mammals. For example, in rodents, an increased incidence of cancer has been noted in the granddaughters and grandsons of mice exposed to DES when pregnant (Newbold et al.,1998; 2000). Similarly, an increased risk of hypospadias has been reported in the sons of women exposed in-utero to DES (Brouwers et al.,2005). Furthermore in rats, deficits in sperm production and infertility have been shown in 3 subsequent unexposed generations, as well as in the first offspring exposed to methoxychlor or vinclozolin while in the uterus of the mother rat. It seems that this is not due to a mutation in the gene, but instead due to altered DNA methylation, which then causes changes in the expression of a gene or genes in a process termed epigenetic reprogramming (Anway et al.,2005). However, the effect with vinclozolin was not found in a subsequent study by industry scientists (Schneider et al.,2008). Nevertheless, transgenerational effects of diet are found (Painter et al.,2008), such that more work is certainly needed to understand how contaminants may affect future generations

section 4
conclusions and recommendations

Conclusions from findings in wildlife

There is very good evidence that pollutant related effects are widespread in male vertebrate wildlife. Some of the most prevalent effects reported in male wildlife, which are associated with pollutants, are related to genital disruption (GD). GD includes an array of manifestations. Notable amongst these are: intersex features (such as egg tissue in the testes of the male); small phallus; small testes; undescended testes or other obvious structural defects of the male reproductive tract; or ambiguous genitals. Genital disruption has been reported in males of some species of wild fish, amphibians, reptiles, mammals, and to a lesser extent, birds. In addition, in egg laying species, including fish, reptiles, and birds, abnormal production of egg yolk protein, VTG, has been recorded in males. All these effects can be caused by exposure to sex hormone disrupting pollutants in the womb, or in the egg. The effects reported show many similarities across vertebrate species, as outlined in Table 1.

More resources are needed to ensure better monitoring of contaminants and their effects in wildlife in locations throughout the globe. There is a need to ensure that wildlife populations are sustainable, and that enough males are contributing to the next generation in order to maintain genetic diversity. Wildlife research is vital not only for wildlife conservation itself, but also to provide insight into potential effects in humans.

Implications for human health

Taken together, the effects seen in wildlife should raise concerns for contaminant induced genital disruption in human male infants. Indeed a condition called testicular dysgenesis syndrome, including birth defects of the penis of baby boys, cryptorchidism (undescended testes), reduced sperm production and testicular cancer, has been suggested, because there is evidence to indicate that these effects may be interlinked in causation (Skakkebaek et al.,2001; 2007; Sharpe and Skakkebaek,2008). Moreover, in many studies these disorders or demasculinization effects have been associated with exposure to certain contaminants or sex hormone disrupting chemicals in humans (Hendersen et al., 1976; Gill et al.,1977; Hosie et al.,2000; Baskin et al.,2001; Swan et al.,2003; Pierik et al.,2004; Swan et al.,2005; Bornman et al.,2005; Hardell et al., 2006; Damgaard et al.,2006; Main et al.,2006; Paris et al.,2006; Main et al.,2007; Fernandez et al.,2007; Andersen et al.,2008; Bonde et al.,2008; Brucker-Davis et al.,2008; McGlynn et al.,2008; Swan,2008). Scientists have also noted that the

rapid pace of the increase of human male reproductive disorders indicates an environmental cause (Skakkebaek et al., 2001), as do studies following baby boys born to immigrants who take on the same risk for testicular cancer, as the offspring of residents born in that country (Myrup et al.,2008). If testicular dysgenesis syndrome is occurring in humans due to pollutants, then genital disruption should be found in wildlife in areas with high levels of pollutants, and this indeed does seem to be the case.

Implications for regulation

Current findings merit urgent action to drastically reduce exposure to chemicals of very high concern, such as EDCs, and persistent and bioaccumulating chemicals. CHEM Trust believes that eliminating exposure and using safer alternatives is the best way forward.

The new EU Regulation on industrial chemicals, called REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), which came into force in June 2007, ensures that chemicals with endocrine disrupting properties can be subject to prior authorisation. This means that such chemicals may have to be phased-out unless they are specifically authorised for a particular use. However, firstly, a Member State or the Commission would have to instigate the drafting of a dossier with a view to bringing the endocrine disrupting chemical on to the candidate list for prior authorisation. Moreover, as it is currently worded, it seems that endocrine disruptors included under the authorisation procedure will be permitted to be used provided that

exposure to the single substance under consideration is below its no effect level. Unfortunately, such an approach would not be protective, because many chemicals to which humans and wildlife are exposed exert oestrogenic or anti-androgenic action, and therefore there is a heightened potential for additive effects from endocrine disrupting chemicals. Even when individual substances are below their so called no effect concentration, derived from experiments on a limited number of rodents, effects could occur in populations at large, due to several substances acting additively. Furthermore, some researchers consider that there may anyway be no threshold for effects for some hormonally active chemicals (Sheehan,2006). Within large populations, it might be conjectured that there will be some who, due to their baseline hormone levels, will experience adverse effects.

There is to be a mandatory review of how EDCs are dealt with under REACH by June 2013. At this review, CHEM Trust believes any further initial authorisations for the use of substances with endocrine disrupting properties should be blocked if there are safer alternatives. In the meantime, it is nevertheless important that Member States and the Commission urgently put substances with these undesirable properties forward for authorisation, even if the current legislative text may allow their use to continue in the short term. This is because the current text of REACH dictates that when authorised substances come up for re-authorisation, if the update of the analysis of alternatives shows that there is indeed a suitable alternative,

a substitution plan must then be put in place.

As of the end of November 2008, the updated EU Regulation on pesticides was not finalised and was still the subject of on-going negotiations. CHEM Trust believes that EU usage of endocrine disrupting pesticides should be phased-out and safer alternatives found, particularly for those which persist in the environment or which give rise to human exposure.

The potential for transgenerational effects also increase the concern, and underlines the need for a more robust approach to regulation. More research is warranted to fully understand the long term effects from exposure to low levels of contaminants, and particularly to understand the science of epigenetics and the impact chemical exposures may have on subsequent generations.

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