

13 Ethinyl oestradiol in the aquatic environment

Susan Jobling and Richard Owen

Many decades of research have shown that when released to the environment, a group of hormones known as oestrogens, both synthetic and naturally occurring, can have serious impacts on wildlife. This includes the development of intersex characteristics in male fish, which diminishes fertility and fecundity. Although often sublethal, such impacts may be permanent and irreversible.

This chapter describes the scientific evidence and regulatory debates concerning one of these oestrogens, ethinylloestradiol (EE2), an active ingredient in the birth control pill. First developed in 1938, it is released to the aquatic environment via wastewater treatment plants. Although it is now clear that wildlife species are exposed to and impacted by a cocktail of endocrine disrupting chemicals, there is also reasonable scientific certainty that EE2 plays a significant role, and at vanishingly low levels in the environment.

In 2004 the Environment Agency of England and Wales accepted this, judging the evidence sufficient to warrant consideration of risk management. In 2012, nearly 75 years after its synthesis, the European Commission proposed to regulate EE2 as a EU-wide 'priority substance' under the Water Framework Directive (the primary legislation for protecting and conserving European water bodies). This proposal was subsequently amended, delaying any decision on a regulatory 'environmental quality standard' until at least 2016.

This is in part because control of EE2 will come at a significant price. Complying with proposed regulatory limits in the environment means removing very low (part per trillion) levels of EE2 from wastewater effluents at considerable expense.

Is this a price we are willing to pay? Or will the price of precautionary action be simply too high — a pill too bitter to swallow? To what extent is society, which has enjoyed decades of flexible fertility and will also ultimately pay for the control and management of its unintended consequences, involved in this decision? And what could this mean for the many thousands of other pharmaceuticals that ubiquitously infiltrate our environment and which could have sublethal effects on aquatic animals at similarly low levels?

13.1 Introduction

A large body of scientific data built up over many decades indicates a cause-effect link between exposure to a complex cocktail of chemicals and the feminisation and demasculinisation of wildlife species, particularly those living in or around the aquatic environment (reviewed in Lyons, 2008). Some scientists also relate exposure to hormonally active chemicals (so-called 'endocrine disruptors', including alkylphenols, bisphenol-A, phthalates, flame retardants and many other chemicals in everyday use) to declining sperm counts, increased incidence of male genital abnormalities, testicular, breast and prostate cancer in human populations (Lyons, 2008; Sharpe, 2009), breast growth in young men (Henley et al., 2007), and early onset of puberty in young girls (Den Hond and Schoeters, 2006; Jacobson-Dickman and Lee, 2009).

Over the last 50 years, since the publication of *Silent Spring* by Rachel Carson in 1962, we have learned that chemicals in food, household products, medicines and cosmetics, can be and are harmful to wildlife at very low levels. Many of these chemicals, each present at vanishingly low (parts per trillion) concentrations, can together have additive biological effects — producing 'something from nothing' in the words of Silva et al. (2002). Awareness of this reality has awoken scientists and the public to an era of possible harms from low-level, chronic chemical pollution (e.g. from human pharmaceuticals).

These chemicals may not have obvious catastrophic effects such as those observed for the endocrine disrupting anti-foulant tributyltin (TBT), which devastated commercially important oyster populations (see EEA, 2001, Ch. 13 on TBT and Gee, 2006). They can, however, have less obvious effects that nonetheless cause irreversible harm to individual organisms. This raises difficult questions concerning the relationship between these damaging but sub-lethal impacts on wildlife and their connections to ecological (e.g. population level) impacts, and to human health. It also raises issues for the precautionary principle, which states that where there is evidence of damage and irreversible harm, lack of full scientific certainty should not excuse inaction (UN, 1992). As we shall describe, the evidence suggests that it is entirely reasonable to invoke the precautionary principle and introduce regulation to limit aquatic exposure to EE2. But this is no trivial matter, and the reasons for this raise serious questions for the precautionary principle itself.

This is the story of one of these chemical pollutants, the contraceptive pill hormone 17 α -ethinyloestradiol (EE2) as it has unfolded in the United Kingdom from the 1970s to today

13.1.1 The contraceptive pill

The first orally active synthetic steroidal oestrogen, EE2⁽¹⁾, was synthesised by Hans Herloff Inhoffen and Walter Hohlweg at Schering AG in Berlin (Inhoffen and Hohlweg, 1938; Maisel, 1965) in 1938. EE2 was invented at a time when scientists across the world were intensifying their research into sex hormones as a means of controlling fertility and gynecological disorders. They were inspired by the remarkable successes achieved during the 1920s in using insulin to treat diabetes, thyroxine to alleviate thyroid deficiencies and metabolic disorders, and by the discovery of progesterone, the ovulation preventing hormone in 1934 (Sneider, 1985).

At a similar time, Edward Charles Dodds, a British medical researcher, discovered the potent oestrogenic properties of diethylstilbesterol (Dodds et al., 1938; EEA, 2001, Ch. 8) and those of bisphenol-A and 4-nonylphenol (Dodds et al., 1936), now well known endocrine disrupting chemicals. While BPA (see Chapter 10 on BPA) and 4-nonylphenol never found uses as drugs (their future was to be in plastics and detergents, respectively), for many medical practitioners and scientists there seemed no limit to the extent to which artificial oestrogens could be put to good medical use. EE2 was first marketed by Schering as Estinyl in 1943 and initially used to manage menopausal symptoms and female hypogonadism. It appeared to be readily absorbed orally and very resistant to degradation and metabolism by the gut. The stability and effects of EE2 paved the way for developing oral contraceptive pills ('the pill'), which eventually occurred in the 1950s and 1960s (Medical News, 1961).

The development of the pill was powerfully intertwined both with concerns about overpopulation and the 'sexual revolution' of the 1960s. Symbolically, it was much more than a tool for contraception. From the start it was linked with the hopes that it could curb population growth and bring about world stability. The first version of the contraceptive pill (Enovid) contained the hormones mestranol (the methyl ether of ethinyl oestradiol) and norethynodrel (a progesterone-like

(¹) 17 α -ethinyloestradiol is the 17 α -ethinyl analogue of the natural female hormone, 17 β -oestradiol.

hormone). As Dr John Rock, a catholic obstetrician-gynecologist who ran some of the first clinical trials of the birth control pill, noted in 1967, 'If taken as it should be ... it will stop ovulation 100 per cent' (CBC digital Archives, 1964).

Today the mechanisms of action of synthetic oestrogens and progestogens ⁽²⁾ are well understood. They are taken up by the cells in the reproductive system, pituitary, bone, liver and other tissues and bind to oestrogen and progesterone receptors, triggering increases or decreases in the expression of genes regulated by these hormones, in turn controlling gender, sexual development and reproduction. When taken correctly in the contraceptive pill, they interfere with the normal monthly cycle of a woman, preventing pregnancy by stopping the ovaries from releasing an egg, making it difficult for sperm to enter the womb (by thickening mucus in the cervix) and making the lining of the womb too thin for a fertilised egg to implant.

The American Food and Drug Administration approved the pill for use in the US in early 1961 and on 4 December 1961, Enoch Powell, then Minister of Health, announced that it could be prescribed through the UK National Health Service at a subsidised price of two shillings per month (Time, 1961). Take up of the pill was fast. Between 1962 and 1969, the number of UK users rose from approximately 50 000 to one million (out of an estimated 10 million users worldwide), generating an enormous social impact and earning it a place on the front cover of Time Magazine in April 1967 (Time, 1967) ⁽³⁾. The pill is hailed as playing a major role in the women's liberation movement and greater sexual freedom (Asbell, 1995; Goldin and Katz, 2002). Its availability, particularly in the developed world, has made a significant and dramatic impact on women's lives: giving unprecedented control over fertility, preventing pregnancy, and so avoiding the mortality and morbidity associated with pregnancy, childbirth and termination.

The pill now comes in 32 different forms and is used by more than 100 million women worldwide. Usage varies widely by country (UNPD, 2006; Leridon, 2006), age, education, and marital status. One quarter of women aged 16–49 in Great Britain currently use the pill — either the combined pill, progesterone-only pill or 'minipill' (Taylor et al.,

2006) — compared to only 3 % of women in Japan (Hayashi, 2004; Hayes, 2009), which, in 1999, became the last country in the developed world to legalise the pill.

13.1.2 Evidence of environmental harm from 'the pill'

In the 1970s some scientists began speculating that using the contraceptive pill might cause environmental problems (e.g. Tabak and Bunch, 1970). They realised that oral medications, including contraceptives, are in fact rather inefficient methods for administering drugs to the body, since it takes a lot of drug administered orally to get a little into the bloodstream. The rest of the medicine passes right through the body and into wastewater in urine and faeces. Since water and waste treatment plants were not designed to remove pharmaceuticals (or indeed other man-made chemicals), it was likely that the contents of our medicine cabinets were unintentionally being passed on directly into the environment, and eventually, even into drinking water supplies.

EE2 is excreted as conjugates of sulphates and glucuronides, along with the natural steroid hormones oestrogens oestrone (E1), 17 β -oestradiol(E2) and oestriol(E3) that occur naturally in humans. The synthetic EE2 shares a common hormonal mode of action with these natural oestrogens, which also means that, when released into the environment, the oestrogenic endocrine disrupting effects of EE2, and the natural steroid oestrogens in combination are additive. EE2, the synthetic oestrogen, is however by far the most potent of the four.

The data supporting the need for risk management of EE2 and the most potent of the three other steroid oestrogens (E2 and E1), due to their endocrine disrupting effects in the environment, are now comprehensive and compelling (Gross-Sorokin et al., 2006; Caldwell et al., 2008). Based on data amassed over many decades, the mechanism of toxicity of EE2, E2 and E1 is now well understood. They are continuously, and widely, released into the aquatic environment and are persistently present, having a half life in fresh water of between less than a day and approximately 50 days under aerobic conditions.

⁽²⁾ Progestogens (also spelled progestagens or gestagens) are a group of hormones including progesterone.

⁽³⁾ Front cover of Time Magazine's April 1967 edition can be viewed at: <http://www.time.com/time/covers/0,16641,19670407,00.html>.

They have been shown to cause endocrine disrupting effects at environmentally relevant concentrations in controlled laboratory studies (e.g. Lange et al., 2001; Nash et al., 2004, reviewed in Caldwell et al., 2008), in field studies of fish placed downstream of sewage treatment works with various types of secondary treatment technologies (Harries et al., 1996 and 1997) and in whole experimental lake studies dosed with EE2 (Kidd et al., 2007).

In some UK rivers, 100 % of wild male fish of the species *Rutilus rutilus* (roach, a common freshwater fish of significance to anglers) sampled between 1995 and 2002 had female characteristics (Jobling et al., 1998) and intersex has now been reported in many fish of a number of freshwater and marine species, in more than 10 countries (Tyler and Jobling, 2008; Hinck et al., 2009). Models predicting exposure of riverine fish to EE2, E2 and E1 in the United Kingdom have also been shown to correlate well with impacts observed in fish populations in the field (Jobling et al., 2006). These impacts damage fish reproductive health, for example affecting fertility and fecundity (Jobling et al., 2002a and 2002b; Harries, Hamilton et al., 2011) and are in some cases irreversible (Rodgers-Gray et al., 2001).

Risk characterisation of EE2 and the other two non-synthetic steroid oestrogens, E2 and E1, in the aquatic environment is possibly one of the most comprehensive for any chemical pollutant. Many millions of euro have been spent on this research over many decades. The chemical industry has concluded that 'endocrine disruption is undoubtedly occurring in wild fish populations' and that the evidence that wildlife has been impacted adversely following exposure to endocrine disrupting substances is 'extensive' (Webb et al., 2003). The pharmaceutical industry, in particular, has carried out and funded some of the key lab studies showing that EE2 plays a key role in causing these effects (e.g. Lange et al., 2001).

In response to this evidence, in 2004 the Environment Agency of England and Wales (EA) concluded that risk management was needed for steroid oestrogens (Gross-Sorokin et al., 2006). In 2012 the European Commission proposed EE2 as a Priority Substance (i.e. a substance requiring control across Europe) under the Water Framework Directive, one of the most important pieces of legislation for protecting European surface and ground waters, proposing regulatory limits in the aquatic environment for EE2. At present, this is only a proposal, with formal processes of

agreement required before regulation can occur. In July 2012 an amendment to this proposal was tabled. This delays review of a proposal for a regulatory limit until 2016 (rather than 2012), which (if adopted) would then have to be complied with by 2027. Nevertheless nearly 75 years after its initial development, during which time some of the most comprehensive and compelling evidence of environmental impact for any chemical has been amassed, a decision to regulate of EE2 is finally under serious consideration. Why has this taken so long? And what lessons can we learn?

The example of EE2 and endocrine disruption in the aquatic environment, and the questions and dilemmas it raises, is in many ways a test case for many thousands of low-level pollutants that infiltrate our environment ubiquitously, many of which have chronic impacts that go beyond the acute polluting effects of past industrial chemicals.

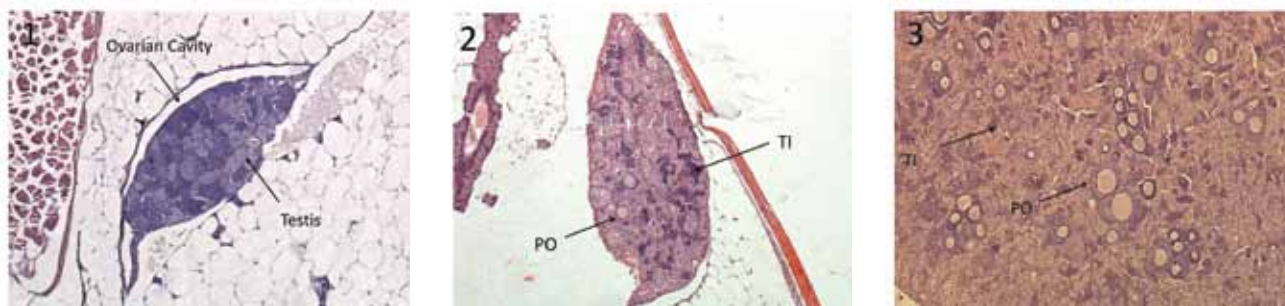
13.2 Early warnings

13.2.1 Early warnings from wildlife: the UK experience

The earliest concerns about possible hormone contamination of water (from contraceptive pills and other sources) related to impacts on male fish reproductive health in UK rivers. In 1978, Tony Dearsley, a Thames water area biologist in the United Kingdom, found eggs developing in the testes of five out of 26 male fish of the commonly caught species *Rutilus rutilus* (the roach) while conducting a routine health check on a small sample of fish from the River Lea.

As Dearsley's manager, Roger Sweeting, then Senior Scientist at Thames Water, noted, 'It was amazing to see macroscopically hermaphrodite fish that were both male and female all at the same time' (Sweeting, pers. comm.). It was not until a year later, however, after reading a paper by Jafri and Ensor (1979), that Sweeting telephoned David Ensor, who confirmed that the findings were highly unusual. Jafri and Ensor had reported a normal incidence of hermaphroditism of 1 in 1 000, in the same species of fish (Jafri and Ensor, 1979), more than 100 times lower than found in the fish collected from the Lower River Lea (see Figure 13.1).

Larger samples of fish confirmed the earlier observations. It was also observed that the incidence of feminisation varied with the age of the fish, with the highest proportion (20 %) found

Figure 13.1 Histological sections showing intersex phenotypes in roach

Note: Image (1) shows a female-like ovarian cavity in an otherwise normal testis. Image (2) shows a severely intersex gonad following exposure to sewage effluent with a testis containing a large number of primary oocytes at a single focus. Image (3) shows the intersex gonad of a wild fish caught from the River Aire, the United Kingdom. TI denotes 'testis lobule'. PO denotes 'primary oocyte'.

in the older fish (aged more than six years). Roger Sweeting reported that this suggested a 'cumulative effect with time' (Sweeting, 1981).

Thames Water, at the time a government-owned company, was proactive in researching the matter further to gain a better understanding of its relevance to public health. It was reasoned that 'some risk of endocrine disturbance in human consumers of the water could be implied' (Sweeting, 1981) as it was known that the River Thames received discharges from numerous wastewater treatment plants (352 according to Williams et al. (2008)) and that it also served as a major potable water source for North London. Water abstracted from the river was purified, used by people, or industry, and then disposed of to sewage treatment works, where it was 'cleaned' before being discharged (as effluent) back into the river, only to be abstracted, used and cleaned a second or subsequent time downstream. (For this reason, it is commonly said that when you drink a glass of water in London, the water has already passed through several pairs of kidneys).

Thames Water's concerns for public health led to further studies (never published) under a research contract given to Liverpool University. Water samples were taken directly from the River Lea at the drinking water abstraction point and transported on a train to Liverpool University, where they were given to rats to drink. Derek Tinsley (a PhD student at the time who now works for the Environment Agency of England and Wales) recalls visiting the station at regular intervals to collect the water. The studies showed clearly that

giving female rats this water to drink for 12 months induced persistent oestrous.

Further studies showed, however, that water samples taken part way through the drinking water treatment process had no effects on the rats. Moreover autopsies of small mammals (water voles and wild rats) trapped around the sewage works revealed no obvious gross reproductive abnormalities. Derek Tinsley recalls that he felt 'relief that there were no signs of any effects of the treated drinking water on the rat reproductive system, and therefore no apparent risks to the consumer' (4).

Thames Water officials felt confident enough to present Tinsley and Ensor's results to a standing committee of the Department of Health, which, after conducting further studies, agreed that there was enough information to discount any possibility of risk to human consumers of water abstracted from the Lower Lea. It is striking that such a rapid decision was made on the basis of this small set of studies. This, as we will go on to discuss, is in stark contrast to decision-making regarding protection of aquatic wildlife from the effects of EE2 and other oestrogens, despite the many studies indicating adverse health effects related to oestrogen exposure.

The possibility that the contraceptive pill hormone might well be causing intersex in fish was not formally stated in any of the scientific reports circulated between the government and the water industry. Nor was any link made with the reports of occurrence of steroidal oestrogens in wastewater, river and potable waters (Tabak and Bunch, 1970;

(4) Personal communication from Derek Tinsley.

Tabak et al., 1981; Aherne et al., 1985). At least one of those studies had recognised that steroidal oestrogens could potentially have adverse effects, even if none were identified:

'the concentrations found are far below therapeutic doses and there appears to be no evidence of adverse effects from reused water resources which may be contaminated from the normal use of such highly active therapeutic agents' (Aherne et al., 1985).

Aherne and colleagues could not have known about the Thames Water studies as they were confidential. Moreover, the significance of their work and the earlier studies by Tabak and colleagues was not recognised, because the human health agencies responsible for international drug regulation at the time usually had limited expertise in environmental issues; consideration of these was not formally required. The situation is today very different, with environmental exposure now recognised as a key consideration (for example in European Medicines Agency guidelines (EMA, 2006) ⁽⁵⁾). Moreover, until the 1990s, any concerted chemical analytical efforts to look for drugs in the environment achieved limited success. This is because the requisite chemical analysis tools (with sufficiently high separatory powers to identify the drugs amid a plethora of other natural and anthropogenic substances in the environment, and sufficiently low detection limits (i.e. nanograms per litre or parts per trillion)) were not commonly available.

Now there is considerable concern about the increasing amounts of pharmaceuticals that are being consumed and found in the environment (Kümmerer, 2004 and 2007; Apoteket AB et al., 2006 and 2009; EEA, 2010; German Advisory Council on the Environment 2007; Mistrapharma, 2011). With an ageing population the UK Office of National Statistics predicts that the country's medicine usage will more than double by 2050 (Nature, 2011).

13.2.2 *More evidence of an environmental problem*

In the mid-1980s, fisheries scientists working for the UK Ministry of Agriculture Fisheries and Food, or MAFF (Dr Colin Purdom, Dr Vic Bye and Dr Alex Scott) were asked to comment on the

evidence collected to date. Quite independently, one of their colleagues, Dr John Sumpter, had found high levels of a female-specific yolk protein (vitellogenin or VTG) in the blood of male fish kept at an experimental fish farm rented by MAFF. VTG is under strict oestrogen control and as males have extremely low (often undetectable) levels of oestrogen in their blood, they can only produce VTG if they are exposed to an oestrogen (Sumpter and Jobling, 1995). The scientists wondered whether the fish were being supplied with water contaminated with oestrogens originating from the treated sewage effluent entering the river upstream of the fish farm, and whether oestrogens in the water might also explain the discovery of feminised wild male fish in the River Lea.

Field trials in the late 1980s, funded by the Department of the Environment (DoE), confirmed that VTG levels in male trout placed for just two weeks in the sewage treatment plant effluent from Rye Meads sewage treatment works (which entered the River Lea) underwent a 100 000 fold increase, reaching levels equivalent to those in mature females. These results provided the impetus for a nationwide survey of effluents (conducted between 1987 and 1990) by Brunel University (John Sumpter and Charles Tyler) and MAFF (Colin Purdom, Vic Bye, Sandy Scott and Peter Hardiman), with funding from the DoE. The results of this survey proved beyond doubt that oestrogenic effluents entering rivers were widespread throughout England and Wales (Purdom et al., 1994).

By the late 1980s, therefore, there was already evidence that wastewater from sewage treatment plants was having harmful effects on aquatic wildlife, and that at least one possible culprit was EE2. This information was not widely circulated beyond government and industrial organisations. Indeed, the results were not published until 1994 (Purdom et al., 1994), because of the contractual agreement between the DoE, MAFF and Brunel University. There was little action: policymakers of that era perhaps preferred to wait until the level of proof linking cause with effect was beyond reasonable doubt, reflecting a wider resistance at the time to precautionary action in the absence of higher levels of proof.

Subsequent research has proven EE2's presence in effluents and natural waters, and established that it

⁽⁵⁾ More recent guidance from the European Medicines Agency states that if the estimated environmental concentration (i.e. predicted surface water concentration) of a medical product is below 0.01ppb and 'no other environmental concerns are apparent' then no further actions are needed in terms of environmental risk assessment, i.e. no action needed by a pharmaceutical company.

is highly likely that it is contributing significantly to the damaging effects seen in wild fish (Caldwell et al., 2009). Moreover, studies carried out in Canada have confirmed large effects associated with very low concentrations in a multi-year study in which fish living in a large experimental lake were exposed to introduced EE2 (Kidd et al. (2007): 6.1 ng/L (+/- 2.8) during the first year, 5.0 ng/L (+/- 1.8) during the second year and 4.8 ng/L (+/- 1.0) during the third year of. This low level introduction of oestrogen provoked a large surge in fish plasma VTG levels, followed by complete collapse of the fish population. The fundamental conclusion, that EE2 and other hormones in wastewater, both natural and synthetic, are harming aquatic wildlife, particularly downstream of wastewater plants with low dilution, has not changed since the late 1980s. Only the level of uncertainty has reduced.

13.2.3 *Widespread endocrine disruption in wild fish and growing evidence of problems in other wildlife*

The discovery in the 1980s that oestrogenic effluents were widespread led logically to more caged fish trials that illustrated the extent of the oestrogenic pollution at greater distances from the sewage treatment works (Harries et al., 1996 and 1997). Extensive field trials in the United Kingdom between 1995 and 2000 also showed unequivocally that intersex in wild roach was widespread and especially prevalent up to 10 km downstream of medium- to large-sized sewage treatment works (serving populations of 50 000 to 675 000) and where dilution of their effluents in receiving river waters was less than 10-fold (Jobling et al., 1998).

In addition, studies on estuarine species of fish illustrated clearly that the effects of oestrogenic contaminants extended beyond the rivers into estuaries (Lye et al., 1997 and 1998; Allen et al., 1999) Feminisation and sub-fertility were also reported in additional wildlife species, especially those living in or around the aquatic environment (reviewed in Lyons, 2008). Specifically:

- amphibians were found to have abnormal production of VTG by males and ovotestes/intersex features;
- reptiles were found to have abnormal production of VTG by males: sex hormone disruption; ovotestes; smaller phallus in alligators and shorter estimated penis length in turtles; decreased hatching; and decreased post hatch survival;

- birds were found to have abnormal VTG production in males; deformities of the reproductive tract; embryonic mortality; reduced reproductive success including egg-shell thinning and poor parenting behaviour;
- otters and mink were found to have reduced penile bone length; smaller testes; and impaired reproduction;
- seals and sea lions were found to have impaired reproduction (including implantation failure, sterility, abortion, premature pupping);
- cetaceans were found to have reduced testosterone levels; impaired reproduction; and hermaphrodite organs;
- polar bears were found to have intersex features and deformed genitals; reduced testes and baculum length; low testosterone levels in adult males; and reduced cub survival.

In none of these cases was a link with exposure to EE2 investigated and/or proven.

13.2.4 *Wildlife as sentinels for human reproductive health*

Beyond the aquatic environment, the feminising syndromes found in wildlife appeared to mirror reports of male infertility, genital abnormalities and testicular cancer observed in the human male population, collectively termed Testicular Dysgenesis Syndrome (TDS; see Box 13.1). If testicular dysgenesis syndrome was occurring in humans due to environmental pollutants, then genital disruption should have been found in wildlife exposed to those pollutants. This did indeed seem to be the case. The question arose whether the effects seen in wildlife and in humans shared a common cause: environmental oestrogens including EE2. There had already been evidence of human health impacts associated with another synthetic oestrogen created in the same year as EE2: diethylstilbestrol (DES). From the 1940s to the early 1970s, pregnant women in the US (and beyond) were commonly prescribed DES in the mistaken belief that it could prevent miscarriage. Some of the sons of these DES mothers developed low sperm counts, undescended testicles and deformations of the penis (Ibaretta and Swan, 2001).

The steepest declines in male reproductive health appear to have taken place in most countries between the 1960s and the 1980s, coinciding not only with the introduction and take up of the contraceptive

Box 13.1 Human health concerns

The first indications that something might be wrong with human sperm came in 1974, when Kinloch Nelson and Bunge produced a small study of the semen quality of men who were about to undergo vasectomies. They found that only 7 % had sperm concentrations above 100 million (Kinloch Nelson and Bunge, 1974), which is well below the 65 % reported earlier by pioneering andrologist, John MacLeod (MacLeod and Heim, 1945).

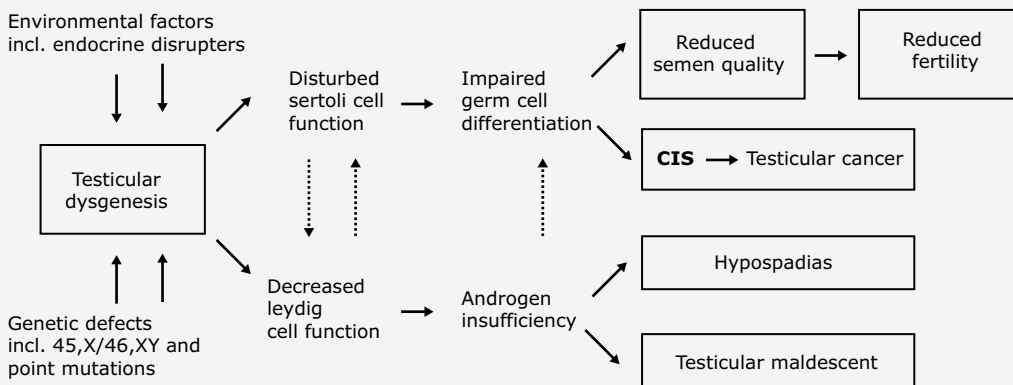
Kinloch Nelson and Bunge speculated that 'an environmental factor to which the entire population has been exposed' might be causing the low sperm counts. These studies were discredited by John MacLeod himself in 1979. While acknowledging a decline in sperm counts in fertile males since the 1930s, MacLeod and Wang (1979) rejected the notion of a larger, overall decline, citing analytical errors in the Nelson and Bunge study and suggesting a concentration of 20 million per ml should be the lower limit for the normal sperm count. This limit was adopted in the WHO guidelines for semen analysis in 1980 (WHO, 1980).

Nothing more was reported until 1992, when Danish clinician Niels Skakkebaek and Elizabeth Carlsen published their ground-breaking paper on studies of sperm counts around the world. In 61 studies going back as far as 1938, they found a decline in average sperm density from 113 million per millilitre in 1940 to 66 million in 1990 (Carlsen et al., 1992). These findings made some scientists wonder whether the human species was approaching a fertility crisis. Debate on this issue was intense and opinion was divided regarding the validity of the apparent fall in global sperm counts (e.g. Swan et al., 1997; Paulsen et al., 1996; Fisch et al., 1996).

The approach taken in Europe (led by Skakkebaek) followed the reasoning that 'if sperm counts have fallen then the average should be lower in men born most recently than they were in the past'. More recent investigations in seven European countries involving more than 4 000 young men have shown that in most of the countries investigated 20 % or more of young men have a subnormal sperm count (less than 20 mn per ml) and the average sperm count is 45–65 million (Jorgensen et al., 2006). This is consistent with sperm counts having fallen, as suggested by the meta-analysis studies. More importantly, it shows that male subfertility is likely to be a common issue for current and future generations, with widespread societal and economic consequences.

The importance of declining semen quality lies partly in its possible link with other problems of male reproductive organs, especially the rise in the incidence of testicular cancer (Adami et al., 1994; Wanderas et al., 1995; Bergstrom et al., 1996; Moller, 2001; McGlynn et al., 2003; Richiardi, 2004) and in congenital malformations of the male reproductive system such as cryptorchidism (undescended testis) and hypospadias (penis malformation) (Hohlbein, 1959; Sweet et al., 1974; WHO, 1991; Matlai and Beral, 1985; Paulozzi et al., 1997; Lund et al., 2009). These diseases often occur together (Prener, 1992; Berthelsen, 1984; Petersen et al., 1999; Schnack et al., 2009) and may have the same underlying pathology — testicular dysgenesis syndrome (TDS) (Sharpe and Skakkebaek, 1993; Skakkebaek, 1998) with a common origin in fetal life (see Figure 13.2).

Figure 13.2 Testicular dysgenesis syndrome



Source: Sharpe and Skakkebaek, 1993.

Box 13.1 Human health concerns (cont.)

Unlike in the case of intersex in fish, there has never been one widely accepted theory regarding the cause of the decline in male reproductive health. Instead, a bewildering array of hypothetical culprits have been posited, including not only the residues of birth control pills in drinking water or inadvertent pill taking during pregnancy, but also a range of anti-androgenic industrial chemicals (male hormone lowering or blocking) and non-chemical stressors that have been shown in laboratory studies to induce TDS when exposure takes place during early pregnancy (Sharpe, 2009). The research in this field has been challenged by the lack of human exposure information and the absence of results of past testing of industrial chemicals for endocrine disruption and other adverse effects that may affect the development of the male reproductive system. Even if animal testing is performed, the results cannot directly be translated to humans because, for example, a chemical may reduce sperm counts by 80–90 % in rats before male fertility is affected.

The net result is that no systematic effort has been made to prevent infertility, despite the substantial potential societal consequences associated with its widespread occurrence. Indeed, the official WHO response to male infertility has been to redefine the ill people as 'normal' by changing the lower reference value for a 'normal' sperm concentration first from 60 mn/ml in 1940s to 20 mn/ml in 1980 and then to the current 15 mn/ml (WHO, 2010), making it of little use in helping society recognise that there is a problem (Skakkebaek, 2010).

pill but also with the entry of many other synthetic chemicals into the environment, the Clean Water Act in 1972 and subsequent upgrading of sewage treatment plants to secondary treatment. Thus, endocrine disruption in the developing child could have occurred through the inadvertent consumption of pills in the first trimester of pregnancy (Smithells, 1981, although refuted by Joffe, 2002). Alternatively, or additionally, it could have resulted from drinking water containing residues of contraceptive pill hormones and a whole plethora of industrial chemicals. Or it could have resulted from inadvertent exposure to these chemicals via application to the skin (cosmetics), inhalation or diet. A more recent report highlights a relationship between modern contraceptive pill use and prostate cancer (Margel and Fleshner, 2011), another hormone-dependent male reproductive disease.

In the case of the contraceptive pill, one might expect the exposure to have been higher in the 1960s, 1970s and 1980s than in later years as the first pill formulations contained up to 100 µg of oestrogen, at least five times higher than current formulations. If exposure to the contraceptive pill was a significant risk factor in causing TDS, the effects should show up in statistics on male reproductive health.

Examination of historical contraceptive pill usage in various countries does indeed suggest higher rates of use (allowing a 30–35 year time lag between exposure of the developing child and the later appearance of testicular cancer) in some countries where rates of

testicular cancer and hypospadias are higher, such as Denmark, Germany, Hungary, the United Kingdom and USA, than in countries where rates are lower, such as Bulgaria, Finland, Italy, Poland, Portugal, Romania and Spain and Japan (Leridon et al., 2006). These associations may well be coincidental, however, as the available evidence in support of the oestrogen theory is not entirely convincing (Raman-Wilms et al., 1995; Toppari et al., 1996; Martin et al., 2008). Indeed, recent animal experimental studies suggest that industrial chemicals that block the action of male hormones (anti-androgens) are more likely culprits of declines in male reproductive health and could even act in combination with oestrogens to induce a proportion of the cases of TDS seen in humans. So far, however, 'there is no human or experimental animal data to support this' (Sharpe, 2009). The lack of cause-effect, especially in human epidemiological studies, could easily be wrongly interpreted as ruling out involvement of endocrine disruptors in TDS. Several factors complicate analysis, including the long latency between exposure and effect and the possibility that the effects may be caused by multiple chemicals in combination, while exposure to each chemical individually may not be insufficient to cause damage. This is also why Bradford Hill's criteria, when invoked in the 2002 WHO report on endocrine disruption (WHO, 2002), rejected the hypothesis of an impact on male reproductive function. These criteria are not suited for determining causation by environmental toxicants because such exposures are simply too complex. Perhaps we should abandon the unrealistic hope of achieving more certainty prior to

policymaking about human male reproductive health and use wildlife as sentinels instead?

The case for endocrine disrupting chemicals causing male sterility in humans and wildlife, showing wildlife were sentinels for human health, was beautifully presented in the British Broadcasting Company's award-winning Horizon documentary 'Assault on the male', written and produced by Deborah Cadbury and screened in 1993. The world began to sit up and take notice. Mounting concern in Europe was such that between 1998 and 2007 the European Commission invested over EUR 150 million into researching endocrine disruption. This research provided the basis for testing both existing chemicals and those planned for introduction to the market in the future for their endocrine disrupting effects. It also furthered understanding of the effects of mixtures of endocrine disrupting chemicals, identification of vulnerable life stages and impacts on male reproductive health. The new data showed that a wide range of chemicals could have endocrine disrupting effects, with a wide range of health impacts.

13.3 The hunt for the culprit chemicals

Against the backdrop of growing global awareness of endocrine disruption, attention focused on the chemicals responsible for the feminised male fish observed in UK rivers. Laboratory studies showed clearly that male fish were extremely sensitive to the presence of EE2 in the water at low ng/L concentrations (Sheahan et al., 1994). Until the mid-1990s, however, few were convinced that this or any other hormone was present in wastewater in sufficient amounts to cause the effects seen in fish.

Coincidentally, during that time information was emerging from the US that, in addition to pharmaceuticals, industrial chemicals in everyday use could mimic oestrogens. Although this was first shown by Charles Dodds in the 1930s (Dodds et al., 1936), widespread awareness of this possibility was instigated by John McLachlan, one of the pioneers of research into environmental oestrogens and the organiser of the first meeting on the topic in 1979 (McLachlan, 1980).

It now seemed possible that effects seen in aquatic and other wildlife were actually more likely to be a result of exposure to cocktails of

'endocrine-disrupting' industrial chemicals, (Clement and Colborn, 1992) than to the contraceptive pill hormone. In 1988 Theo Colborn, in her research on the environmental condition of the North American Great Lakes, showed that persistent, man-made chemicals were being transferred from top predator females to their offspring, undermining the construction and programming of their youngsters' organs before they were born. In 1991, Theo convened a meeting ('The Wingspread Meeting') of 21 international scientists from 15 different disciplines to share relevant research on the topic and it was during this meeting that the term 'endocrine disruption' was coined.

It was also in 1991 that Dr Ana Soto published a paper about the oestrogenic effects of nonylphenol, a chemical compound used in manufacturing a large group of industrial detergents (Soto et al., 1991). Some detective work by Susan Jobling (then a student of John Sumpter) at Brunel University revealed that the environmental chemist Walter Giger had identified these chemicals in sewage treatment works effluents, sewage sludge and river water (Giger et al., 1984) at concentrations that Jobling later confirmed were oestrogenic to fish exposed to the chemicals in the laboratory (Jobling and Sumpter, 1993; Jobling et al., 1996).

An earlier report of the oestrogenic activity of 4-nonylphenol, was published in 1936 by Charles Dodds when he was trying to synthesise one of the first synthetic oestrogens, diethylstilbesterol⁽⁶⁾. The detergent industry may have been unaware of this literature when it embarked on the largescale manufacture and sale of nonylphenol ethoxylates as detergents in the 1940s, leading to the contamination of many rivers and estuaries with these oestrogenic chemicals.

While the causal links between exposure to industrial chemicals and endocrine disruption in most wildlife species were still unclear in the 1990s, further research in the United Kingdom and other European countries showed that nonylphenolic chemicals were causing at least part of the problem in wild fish in some, but not all, areas (Sheahan et al., 2002a and 2002b). However, more sophisticated studies employing chemical fractionation and screening of effluents using an *in vitro* oestrogenicity screen showed that naturally occurring and synthetic steroid oestrogens (EE2, E2

⁽⁶⁾ Interestingly, Dodds identified another controversial compound at the same time — the environmental endocrine disruptor bisphenol-A (Dodds, 1938). See also Chapter 10 on BPA.

and E1) were in fact the most potent oestrogenically active substances present in domestic effluents (Desbrow et al., 1998; Routledge et al., 1998; Snyder et al., 2001), responsible for much of the oestrogenic activity found in wastewaters and rivers throughout most of the world. Of these, EE2 was by far the most potent: the pill was once again under the spotlight.

13.4 Government and industry action in the 1990s

Enormous efforts were made in the 1990s to assess and manage the risks of alkylphenols, not only because of their endocrine disrupting effects but also their wider toxicity to aquatic life:

Following recommendations of the UK Chemicals Stakeholder Forum, the UK government negotiated a voluntary agreement with the suppliers of nonylphenols, octylphenols, and their respective ethoxylates. Suppliers thereby agreed not to promote octylphenol (another endocrine disruptor also found in sewage effluent) as a substitute for nonylphenols, not to manufacture or import new formulations or products containing those substances, and to reformulate existing products to remove those substances as a matter of urgency. This was a constructive application of the precautionary principle (Lokke, 2006), which has not been extended to EE2.

Similarly, in mainland Europe, the European Union undertook a risk assessment of nonylphenols and, as a result, restrictions on using nonylphenol have been imposed across Europe.

At the same time, continued uncertainty about exposure to chemical pollutants in the environment and their effects on the human population (particularly regarding reproductive health) were highlighted during discussions at a major European workshop at Weybridge, the United Kingdom (?), on endocrine disrupting chemicals held in December 1996 and jointly sponsored by the European Commission, the European Environment Agency, the European Centre for Environment and Health and the WHO (EU, 1996). An EU strategy on endocrine disruptors was launched in 1999 to begin to address the problem (EU, 1999) but still no action was taken on EE2.

13.5 The last decade of research

Since the mid-1990s, oestrogenic sewage-treatment works effluents have been identified more widely across Europe, and globally, e.g. in Denmark (Bjerregaard et al., 2006), Germany (Hecker et al., 2002), the Netherlands (Vethaak et al., 2005), Portugal (Diniz et al., 2005), Sweden (Larsson et al., 1999), Switzerland (Vermeirssen et al., 2005), China (Ma et al., 2005), Japan (Higashitani et al., 2003) and the United States (Folmar et al., 1996). More extensive evidence has also emerged from around the world showing widespread endocrine disruption in fish in rivers (Hinck et al., 2009; Bjerregaard et al., 2006; Blazer et al., 2007; De Metrio et al., 2003; Hinck et al., 2009; Penaz et al., 2005; Vajda et al., 2008), estuaries (Allen et al., 1999) and oceans (Cho et al., 2003; Ohkubo et al., 2003; Fossi et al., 2004; Kirby et al., 2004; Scott et al., 2006 and 2007).

In general, the situation in other countries appears to match that found in the United Kingdom. The incidence and severity of endocrine disruption appears to relate largely to the size of the sewage works (treatment type is also important), most importantly, and the dilution of its effluent in the receiving water. This leads to generally lesser effects in fish inhabiting large rivers with high dilution factors (a common scenario in the US and parts of Europe, for example) compared with those in smaller rivers with little dilution (a common scenario in the United Kingdom and other parts of Europe). In Japan, where contraceptive pill hormone use is probably the lowest anywhere in the developed world and where endocrine disruption in fish is reported to be quite rare (Tanaka et al., 2001; Higashitani et al., 2003), the greater mean river flow, and hence available dilution per capita (five times more than in the United Kingdom), suggests that combined steroid estrogen potency will be less across its rivers than in the United Kingdom. Even if the Japanese population were to take up the contraceptive pill to the extent of use in England then widespread endocrine disruption in fish would still not be predicted because of the large amount of available dilution (Johnson et al., 2012).

A growing body of evidence also shows that the harm caused by exposure to endocrine disrupting chemicals early in development is, in many cases, irreversible. In fish, as in humans and rodents, feminisation of the male reproductive tract occurs early in development and produces a fish with

(?) In 2006 the 10th anniversary of Weybridge was marked with a conference organised by the Academy of Finland, the European Commission (DG Research), and the EEA. The EEA has updated and published the papers from that meeting (EEA, 2012).

both an oviduct and a sperm duct. Depuration in clean water does not correct this condition, indicating that feminised ducts seen in wild roach are likely to be a permanent feature (Rodgers-Gray, 2001). Considerable evidence also shows that both the prevalence and the severity of feminisation in wild roach increases with age (Jobling et al., 2006), in extreme cases resulting in a 100 % female population after three years of continuous exposure to treated sewage effluents or EE2 (Lange et al., 2009).

Population-relevant effects of EE2 have also been documented, including a complete fish life cycle test carried out by the original manufacturers of EE2, Schering (Lange et al., 2001), which showed inhibition of breeding at concentrations exceeding the environmentally relevant concentration of 2 ng/L and full sex reversal of males producing an all female population at 4 ng/L. This effect was corroborated by Kidd et al., (2007), who (as we have already noted) dosed an entire lake with EE2, causing a population collapse at concentrations of approximately 5–6 ng/L.

Improvements in analytical power, lower analytical instrument detection limits and innovative modelling approaches have led to the discovery of other steroid oestrogens in the environment, such as equine oestrogens used in hormone replacement therapies (Tyler et al., 2009). There are now also more accurate measurements (Williams et al., 2003; Kanda and Churchley, 2008) and credible modelled estimates (Hannah et al., 2009) of steroid oestrogen concentrations present in sewage effluents and in rivers. This has caused a mixture of doubt and amazement at the possibility that EE2 could be causing endocrine disruption at the very low concentrations at which it is present.

The last decade has also brought the realisation that risk assessments for mixtures of hormonally active chemicals are not adequately protective if based on data for individual substances. Mixtures of synthetic and natural oestrogens (EE2, E2, E1) each at or below their individual 'no effect' concentration were shown to be particularly potent when present in combination (Silva et al., 2002; Thorpe et al., 2003; Brian et al., 2005 and 2007) or with other industrial chemicals with anti-androgenic (male-hormone blocking) activity. These findings confirmed concerns originally mooted by Rachel Carson in 1962 in her book, *Silent Spring*.

More recent reports show an almost concurrent incidence of anti-androgenic chemicals and oestrogens in treated wastewater throughout the

United Kingdom. Statistical modelling of exposure and effect data suggest that these chemicals (although not yet identified) could play a pivotal role in causing feminising effects in male fish in UK rivers. Until now it was thought that such effects were caused only by oestrogens found in contraceptive pills and some industrial chemicals (Jobling et al., 2009).

There may be a further twist in the story. Synthetic progesterones (which complement synthetic oestrogen in the contraceptive pill) have been identified in natural waters (Standley et al., 2008; Kuster et al., 2008) and reported to cause effects in fish when present at ng/L concentrations (Paulos et al., 2010; DeQuattro et al., 2012). Ironically, despite the history of their combined use, oestrogens and progesterones have yet to be tested in fish 'in combination', despite the fact that human females have been carrying out this 'test' for 50 years.

13.6 From risk assessment to risk management

In 2004, some 25 years after initial observations of intersex in fish, the UK government accepted the weight of evidence that EE2, E2 and E1 in combination posed a significant risk to aquatic life through their endocrine disrupting effects (Gross-Sorokin et al., 2006). The long journey to this point was passionately championed by Geoff Brighty, Science Manager at the Environment Agency of England and Wales. His team, in collaboration with others, built and defended the evidence-based case against steroid oestrogens and other endocrine disrupting chemicals in UK rivers.

As Brighty remarked in 2004, 'We now have enough data to act as a policy trigger for taking action' (ScienceBlog, 2004).

End-of-pipe treatment of effluent by water companies was chosen as the risk management approach, in comparison to alternative approaches (such as pharmaceutical industry action to develop substitutes for the active ingredient (EE2) in the pill). This may have partly reflected awareness of the public health benefits of the oral contraceptive, the fact that both naturally-excreted and synthetic oestrogens posed risks via their endocrine disrupting effects (i.e. that both would need to be removed) and that other priority hazardous chemicals would also be reduced with the end-of-pipe treatment approach.

This would place the responsibility for risk management on the water industry and (ultimately) the cost of treatment of the tax paying public. In

2007 the Environment Agency began to develop a (draft) technical environmental quality standard (EQS) — a target concentration which could be used for regulatory compliance for EE2- based on a predicted no effect concentration of 0.1ng/L (Young et al., 2004, Figure 13.3). The Environment Agency also identified the EU Water Framework Directive (WFD) as an appropriate legislative mechanism within which the EQS could be enforced. Options for control under the WFD might be to propose EE2 either as a nationally important 'specific pollutant', or an EU-wide 'priority substance' requiring control across Europe. The WFD is the most important EU legislation for managing water resources and had already been used to regulate other endocrine disrupting chemicals such as TBT and nonylphenol. Since additional endocrine disrupting chemicals were likely to be proposed as 'specific pollutants' or 'priority substances' in order to decrease their overall environmental burden, this was a reasonable option for the steroid oestrogens. At this point, no other country in the world had Environmental Quality Standards for any of these substances⁽⁸⁾.

Before introducing risk management of this type there is an important step known as risk evaluation, which quantifies the wider consequences and costs to society of the proposed management approach and balances them against the benefits. The various options on how to proceed are then evaluated and a decision made. For example, a regulatory impact assessment may be undertaken to evaluate the implications of bringing in regulation and implementing an EQS.

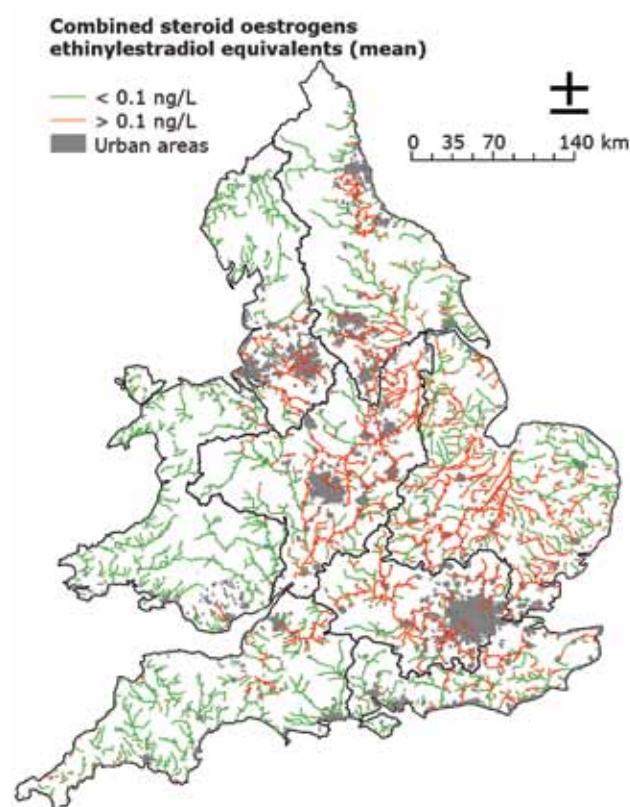
In the case of EE2 and the other two steroid oestrogens it was necessary to understand the efficiency of various treatment approaches in removing these substances from sewage treatment plant final effluents. The approaches included both existing and new (advanced) treatment methods: these would need to be quantified in terms of both financial and carbon costs.

In 2004 the UK water industry, in collaboration with the Environment Agency and the UK government, and under the watchful eye of the independent auditor Ofwat, commenced a comprehensive and lengthy work programme to address this goal. The so-called 'National Demonstration Programme' (Gross-Sorokin, 2006)

was budgeted at GBP 25–40 million and was not welcomed by the UK water industry, which financed most of the costs. All ten water companies in England and Wales were involved.

In the first phase of the programme fourteen sewage treatment plants were used to evaluate the efficiency of oestrogen removal through 'conventional' treatment technologies. These consisted of primary or chemically aided primary sedimentation with secondary treatment by nitrifying, non-nitrifying activated sludge (ASP) or biological filtration and in some cases tertiary sand or biologically aerated filtration. In the second phase, two so-called 'full-scale' plants were used to evaluate the most promising new technology as an additional

Figure 13.3 UK rivers and streams exceeding the Predicted No Effect Concentration (PNEC) for EE2



Note: In rivers and streams coloured red the suggested Predicted No Effect Concentration (PNEC) for EE2 of 0.1 ng/L is exceeded. In rivers coloured green the combined concentrations of steroid oestrogens (in EE2 equivalents) are less than the suggested PNEC.

⁽⁸⁾ In the US, for example, the FDA regulates pharmaceutical manufacture and the EPA regulates discharge under the Clean Water Act. However, these limitations may not be strict enough to protect the environment because they are technology-based and do not rely on environmental data. As explained later, there is no treatment technique currently available that comprehensively deals with EE2.

advanced tertiary effluent treatment: granular activated carbon (GAC).

The first phase of the Demonstration Programme was completed in mid-2008 (UKWIR, 2009). Results showed that while existing treatment approaches were effective for removal of oestrone and oestradiol, particularly using nitrifying activated sludge treatment, EE2 was far harder to remove. The most efficient treatment, nitrifying ASP, removed some 54 %, with tertiary treatments accounting for a further reduction of 0–38 % of that remaining, depending on the form of tertiary treatment.

These results are completely in line with the consensus seen in current international scientific literature, as comprehensively reviewed by Racz and Goel (2010). Wastewater treatment plants with long retention times (greater than 10 days), especially those performing nitrification, seem to be generally more effective at removing oestrogens because they allow the enrichment of slow-growing bacteria, such as nitrifying bacteria, and the establishment of a more diverse ecological community including species capable of degrading EE2. For this reason, expensive membrane bioreactors with microfiltration and ultrafiltration as well as long retention times have been shown to effectively degrade and reduce the concentrations of oestrogens in effluents, including EE2.

In general, however, EE2 is not nearly as easily biologically removed as the other oestrogens. The ethinyl group of EE2 is thought to hinder EE2 sorption and metabolism. Furthermore, EE2 often exists in the aquatic environment at concentrations below those at which a substrate can support bacterial growth. Biodegradation studies at EE2 concentrations greater than those found in natural environments (mainly conducted at such concentrations due to limitations in analytical chemistry techniques) may therefore lead to erroneous conclusions about the occurrence, rates and products of microbial transformation of EE2 and the other steroid oestrogens.

As a consequence of removal inefficiencies, the proposed EE2 Predicted No Effect Concentration (PNEC) of 0.1 ng/L is exceeded in many UK final effluents entering the aquatic environment, irrespective of conventional treatment type (see Figure 13.3). The results of the second phase of the UK Demonstration Programme were reported in May 2010 (NDP, 2010). They showed that additional treatment using a novel approach called granular activated carbon (GAC) can be effective at removing EE2, producing final effluents below the EE2 PNEC

and with no significant induction in fish VTG or intersex (Filby et al., 2010; Baynes et al., 2012). GAC could therefore offer the most promising route for preventing EE2's entry into the aquatic environment at harmful levels (noting that in one study reproduction in fish was slightly, but significantly impacted by effluent subjected to GAC treatment). The Demonstration Programme showed, however, that GAC suffers from a fundamental problem: it is expensive to implement.

For a small town with a 50 000 population equivalent (PE) sewage treatment plant, the capital costs alone of setting up additional GAC were calculated as being over EUR 3 million. That rose to over EUR 8 million for a 250 000 PE works serving a large town such as Swindon, which was one of the sites chosen in the United Kingdom for assessing the technology. Operating costs per annum were calculated as being EUR 800 000 for a 250 000 PE sewage treatment plant, but this would depend on the life of the granular activated carbon. Costs were calculated to approximately 14 kg of CO₂ per person per year. Provisional estimates by the UK government showed that, in total for England and Wales, this would translate into costs of between EUR 32 and 37 billion for the approximately 1 360 sewage treatment works that would require additional treatment (Owen and Jobling, 2012). Again, these findings are in line with the findings of Racz and Goel (2010), who concluded that 'Much attention has also been placed on studying methods of removing oestrogens prior to discharging effluent or disposing waste sludge. While advanced treatment systems such as chemical removal, activated carbon, chlorination, ozonation, ultraviolet irradiation, membrane separation, and other novel approaches may be effective, their current capital and operation costs may make them not viable options.'

Faced with this information, those tasked with implementation of an EQS for EE2 (for example as a 'priority substance' under the Water Framework Directive, see below) increasingly began to worry about two important considerations: technical feasibility and disproportionate cost. It confirmed what they had suspected for many years: EE2 was potent and hard to get rid of.

Evaluation of risk management costs, which have to be calculated at a national scale given the widespread nature of endocrine disruption (Figure 13.4), is however only one half of the cost benefit equation. They must be balanced against the (often intangible) benefits of risk management to key stakeholders, such as the angling community,

Figure 13.4 Extent of sexual disruption in roach in English rivers

Note: Intersex was present at 44 (86 %) of 51 sites surveyed, with an aggregate incidence of intersex of 23 % of sampled males. Coloured symbols indicate incidence of intersex at the different river sites surveyed.

Source: Taylor et al., 2005.

and the public, who will ultimately have to pay for it. This has been a highly contested and often acrimonious area of debate, with highly charged discussions about what constitutes harm at the level of individual fish and what it means for (more ecologically important) fish populations. Adding to this debate is the fact that the costs associated with removing EE2 and E2 should not be seen in isolation: treatment such as GAC would also quite possibly serve to reduce/remove other substances posing risks to the environment and requiring control. Why should such removal costs not be considered for all such chemicals as a whole: is it scientifically incorrect to blame just EE2 for the costs?

Another consideration is how such costs might scale at an EU-wide level, where EU-wide regulation might occur. One European Commission estimate for this is EUR 11–18 per person per annum (EC, 2012). EUREAU (European Federation of National Associations of Water and Wastewater Services) however estimates that these costs are much higher; 25–50 % of the current annual

sewerage charges per year (EUREAU initial position paper on amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy). There is clearly considerable uncertainty here.

The costs at an individual country level are likely to vary on a country by country basis, for example varying with the population density, status of wastewater treatment and size of rivers. In some cases individual country costs may be substantially lower than those estimated for the United Kingdom (e.g. see EU project Neptune and the Swiss project Micropoll; Eawag, 2009). Many European countries have lower population densities and much greater dilution of effluents in their receiving rivers than seen in the United Kingdom. Consequently, the total national costs of complying with any regulation concerning EE2 could be considerably lower in these countries than in the United Kingdom. In addition, the quality of treatment plants in mainland Western Europe is in general higher than in the United Kingdom. In Germany, for example, most sewage treatment plants have three stages and some even have a fourth stage, whilst in the United Kingdom, two stages are most commonly encountered. As we have already noted, the addition of a third stage, whether it be GAC, mild ozonation or simple sand filtration could cause dramatic reductions in estrogen concentrations and their biological effects on fish. As recently demonstrated by Baynes et al. (2012) using biological effects rather than chemical concentrations as measures of effective risk reduction, sand filtration following activated sludge treatment was almost as effective as GAC at preventing the feminisation of male fish albeit it was two thirds cheaper.

For a number of years the decision of whether or not to regulate EE2 appeared to stall in the still waters of cost-benefit analysis, with little chance of resolution and little movement forward. Then, in January 2012, the European Commission proposed a revised list of 'priority substances' for the Water Framework Directive (EC 2012). This included the oestrogens 17 β -oestradiol and 17 α -ethinyloestradiol. The proposed regulatory EQS for EE2 was 0.035 ng/L for inland surface waters (e.g. rivers and lakes): this is the annually-averaged limit in these water bodies. If this proposed EQS is adopted (a first vote on which occurs in the European Parliament's Environment, Public Health and Food Safety Committee in November 2012), it could be taken into account in the 2015 updated River Basin Management Plans and associated 'Programmes of Measures' across Europe, with enforcement required by 2021. But even now, nearly 75 years after its

initial manufacture in 1938, and decades of research concerning its environmental endocrine disrupting effects, this remains a proposal requiring agreement: the decision to regulate or not is yet to be made and may be stalled by recent representations to the EC from both the water and pharmaceutical industries (EUREAU, 2012).

Indeed in July 2012 an amendment to this proposal was tabled, stating 'It is appropriate not to specify the EQS for certain substances of pharmaceutical relevance that have been added to the list of priority substances' and that 'The Commission should propose the EQS for these substances in the next review of the list in 2016, and appropriate measures should be introduced...with the aim of meeting the EQS by 2027' ⁽⁹⁾.

13.7 Late lessons

Ethinylloestradiol, the active ingredient in the birth control pill, has allowed women to control their fertility reliably on a global scale. But it has come at a price to the environment. As mixtures, EE2 and other oestrogens, both synthetic and natural, have been shown to have serious impacts on wildlife — impacts that can be associated with early life exposure but manifest themselves later in adult life. Such impacts are often sub-lethal but may be permanent and irreversible. They may also serve as sentinels for impacts on human health via environmental oestrogen or other endocrine disrupting chemical exposure.

Since wildlife is exposed to a cocktail of endocrine disrupting chemicals, it is naive to conclude that EE2 alone is the culprit. It is, however, the most potent oestrogenic of the steroid oestrogens, occurring widely in effluents entering the environment, at concentrations that can frequently exceed the Predicted No Effect Concentration of 0.1 parts per trillion (UKWIR, 2009). There is reasonable certainty, based on sufficient scientific evidence, that EE2 plays a significant role in causing the reproductive health impacts observed in fish. The need for risk management has been accepted by both the Environment Agency of England and Wales (Gross-Sorokin et al., 2006) and by the European Commission through its proposal for EE2 to become a priority substance requiring control (EC, 2012).

But this is a story that is far from over, and is one that continues to raise important questions that will apply not only to EE2 but to any other pollutants exerting damaging but often sub-lethal effects at very low concentrations. For a decision to regulate EE2 has not been agreed. A critical question is whether we as a society are willing to pay a potentially very high premium to be precautionary and exclude EE2 from the environment.

Alternatively, would we, as a society, prefer to live with the impacts of EE2 and other oestrogens on wildlife; are they acceptable risks? To what extent is society, which ultimately bears the benefits of flexible fertility but also the costs of cleaning up its unintended consequences on the environment, having a say on this decision?

This historical retrospective allows us to identify several important lessons, which are central not only to regulation of EE2 and other steroid oestrogens but also for many other low-level chemical pollutants in the environment with sublethal effects, alone or in combination, now and in the future.

13.7.1 Lesson 1: for low-level pollutants in the environment, is the price of being precautionary simply too high?

With regulation of EE2 now a serious proposal in Europe, the water industry, regulators and national governments are faced (as will be the case with many other low-level pollutants) with risk management that will be a costly process if EE2 is to be removed from sewage treatment works final effluents to the vanishingly low levels that will be required for compliance. The target level proposed by the EC for EE2 as an annual EQS has been set at 0.035 ng/L for inland surface waters (e.g. rivers and lakes). This is the regulatory concentration *in the water body itself*, not in the final effluent discharged from the sewage treatment works. However, as the UK Demonstration Programme has shown, reducing levels of EE2 in final effluents via end-of-pipe treatment to enable compliance with this EQS (e.g. for those water bodies with low dilution receiving large volumes of oestrogenic effluent) will be extremely difficult. The most promising technology, granular activated carbon, might achieve this, but it is expensive and may have a potentially large carbon footprint. Mild ozonation may also be

⁽⁹⁾ Draft report on the proposal for a directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy (17.07.2012). Proposer Richard Seeber, European Member of Parliament.

effective, but this will still be expensive in terms of capital set up costs ⁽¹⁰⁾.

Measuring compliance with the low EQS for EE2 and E2 will also be a significant challenge. The techniques available to measure such very low levels of EE2 in natural waters and effluents are hardly routine. Some efforts have been made to develop more amenable enzyme-linked immunoassay analysis methods but these have been fraught with issues around selectivity and sensitivity at the very low levels of detection required. This leaves both the water industry and regulators with a fundamental problem: it will be costly to remove EE2 to enable compliance with the target EQS, and costly to monitor and demonstrate legal compliance itself. So while it is technically possible to develop an EQS, in practice it may be very hard to implement. The widespread nature of contamination has only added to the problem: this is unlikely to be an issue in one isolated location, but one with significant cost implications across Europe and beyond.

What other options might there be? Since EE2 is both the most potent of the steroid oestrogens and, seemingly, the most difficult to remove by conventional sewage treatment, could there be an argument for substituting this as the active ingredient in the contraceptive pill, leaving the remaining oestrogens to be removed conventionally at less cost? But what would replace it? If there were sufficient demand or need, the pharmaceutical industry could continue to provide women with access to birth control and reproductive choice while substantively altering the design of pharmaceuticals to protect the environment from unnecessary harm. This would represent a constructive precautionary approach ⁽¹¹⁾. Is this option preferable?

13.7.2 Lesson 2: low-level pollutants with sublethal effects present fundamental issues for the precautionary principle

A number of definitions and interpretations of the precautionary principle exist (see Weiner

and Rogers, 2002; Gee, 2006), of which the Rio Declaration (UN, 1992) is one that is widely cited:

'Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation' ⁽¹²⁾.

The chapter on the precautionary principle (PP) in this volume, provides different definitions of the PP taken from other international treaties and the European Court of Justice, including the working definition proposed by the EEA which is designed to improve common understanding about the meaning and application of the PP.

However, for this chapter it will be useful to consider the constituent parts of the Rio definition, the first of which deals with threats of serious or irreversible damage.

It is not in dispute that intersex in fish represents both serious and irreversible damage to fish and that exposure to environmentally relevant levels of EE2 has adverse effects on fish reproduction, an ecologically relevant measure of impact. Uncertainties remain, particularly around fish population level effects, but the precautionary principle aims to promote action in the face of such uncertainties, when there is evidence of serious environmental damage that is irreversible. In fact, the level of scientific certainty concerning the risks (or threats) posed by oestrogens as mixtures to aquatic life, mediated through their endocrine disrupting effects is extremely high and there seems to be little or no doubt that there is sufficient evidence to justify applying the precautionary principle (Gross-Sorokin et al., 2006). Responding to this evidence of harm, the water and chemical industries (and indeed some scientists) have increasingly asked the question, 'so what?' There may be male fish with eggs in their testes and this might be unpleasant, irreversible and widespread. But does it seriously damage fish populations? (Webb et al., 2003). Why pay potentially vast sums

⁽¹⁰⁾ It should be noted that some European countries have decided to clean up wastewater, even in the absence of regulation of EE2 and E2, but in recognition of contamination of wastewater by pharmaceuticals, pesticides and endocrine disruptors. Full-scale treatments will be installed on more than 100 Swiss WWTP treating about 80 % of the Swiss municipal wastewater. The first full-scale ozonation plant will go in operation in Spring 2013 and will cost only 5 EUR/person/year including both capital and operational costs, because sand filtration already exists on this plant.

⁽¹¹⁾ There has been some research towards development of contraceptive formulations such as the progesterone-only pill, which do not contain oestrogen and instead target the female reproductive cycle more specifically (Lakha et al., 2007).

⁽¹²⁾ A European Commission Communication on the Precautionary Principle (EC, 2000) sets out guidance on when to apply the precautionary principle e.g. 'where there are reasonable grounds for concern'. The Communication sets out a set of general principles underpinning application of the PP This includes 'examining costs and benefits' which 'entails comparing the overall cost to the Community of action and lack of action, in both the short and long term. This is not simply an economic cost-benefit analysis: its scope is much broader, and includes non-economic considerations, such as the efficacy of possible options and their acceptability to the public' (p. 4). It also includes the need for the decision-making process to be 'transparent'.

of money for risk management when the seriousness of such population — level effects is uncertain? These populations might collapse in the future, as indicated in the Great Lakes study of EE2 (Kidd et al., 2007), but maybe they will not. Is intersex an unpalatable but acceptable harm?

But even if the seriousness of the harmful threats is accepted, the precautionary principle's Achilles heel, however, as defined in the Rio Declaration, lies in the words 'cost effective'. The central issue for oestrogens (and quite possibly for many other chemicals that cause sublethal impacts at very low levels) is that any risk management measures are likely to be very costly, and as we have discussed above, this may be a price too high to pay.

The potentially high costs of risk management have combined with protracted debates about what constitutes acceptable harm to seriously delay decision-making about the regulation of EE2 and other steroid oestrogens. This has been compounded by a precautionary principle whose definition includes issues of disproportionate cost and cost effectiveness, either explicitly or implicitly⁽¹³⁾. Defined in this way the precautionary principle may be logical and rational, but it can also paradoxically become a perfect excuse for inaction or, at best, seriously delayed action. It is therefore perhaps no surprise that eight years after the UK government officially recognised EE2 and other steroid oestrogens as posing a risk to wildlife that should be managed, and some 30 years after the first observations of their effects in wild fish populations, that the regulation of EE2 is still undecided. And even if the EC proposal is agreed, it will not come into force until at least 2015.

Such long delays have, one might argue, been completely within the spirit of the precautionary principle at least as defined in the Rio declaration. The precautionary principle is well intended and should expedite decision-making in the face of uncertainty. But in reality decision-making has been painfully slow. We are left with the uncomfortable knowledge of a serious environmental issue that has been, and continues to be, unresolved: one which we may have to live with if the price of precaution is deemed too high. This is a bitter pill to swallow.

13.7.3 Lesson 3: the need for an open debate on precaution and decision-making

Our environment is full of low-level pollutants present as mixtures that cause sublethal effects. The European Inventory of Existing Commercial Chemical Substances (EINECS) lists over 100 000 chemical compounds and little is known about the toxicity of about 75 % of them. Several hundred new substances are marketed each year after some basic premarket toxicity testing and these are registered in the European List of Notified Chemical Substances (ELINCS), which currently contains about 2 000 chemicals.

In 2007 the new chemicals EU regulation, REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) was enacted. This reformed chemicals laws and set up procedures and responsibilities to address the backlog of untested chemicals, focusing on some 30 000 substances now being evaluated by industry and the new EU chemicals agency ECHA. Hazard identification and quantification is challenging however, and implementing monitoring programmes and conducting risk assessments for this whole 'chemicals universe' is unfeasible. Moreover, we do not have the tools to fully analyse how mixtures of these chemicals behave. The only logical way forward seems to be to reduce exposure as much as possible — to be precautionary. But this comes at a price and raises ethical questions of where responsibility should lie. Do we want the water industry to reduce exposure through costly treatment? Do we want the pharmaceutical industries to invest in developing new, less harmful contraceptive pills? Either way, are we as a society, prepared to pay for it? Do we care?

The average fish in a stream or person in the street now has hundreds of novel compounds in their bodies that were not there 60 years ago. We can measure them in adult and foetal tissue. We know they have detrimental effects such as intersex in fish. We have changed the chemical environment of the developing organism. EE2 is a perfect case study of how we are responding as a society to difficult decisions regarding the need for, and challenges of, risk management for these low-level pollutants with chronic sublethal effects.

⁽¹³⁾ The EU communication on the precautionary principle also recommends an 'Examination of the benefits and costs of action and lack of action'. This 'examination of the pros and cons should include an economic cost benefit analysis where this is appropriate and possible. However, such an 'examination of the pros and cons cannot be reduced to an economic cost-benefit analysis'. It is wider in scope and includes non-economic considerations (EC, 2000). The EEA working definition of the PP also uses the broader 'pros and cons' rather than 'costs and benefits' for similar reasons, including the importance of the non-quantifiable 'cons' such as the melt down of public trust in scientists and politicians which occurred in the BSE saga (see EEA, 2001, Ch. 15 on BSE).

A key observation from this case study is that the public has been and continues to be silent witnesses. A key lesson learnt from previous case studies (EEA, 2001; Gee, 2006; Lokke, 2006) has been that the process of applying precaution must encourage public participation, such that the costs of action (e.g. risk management) and potential costs of inaction are debated. This enables value judgements and decisions to be made in an open and democratic way. Transparency of decision-making and the need to involve all interested parties as early as possible is a central tenet of the European Commission communication on the precautionary principle (EC, 2000), which states that:

'All interested parties should be involved to the fullest extent possible in the study of various risk management options that may be envisaged once the results of the scientific evaluation and/or risk assessment are available.'

The US National Academy of Sciences has also repeatedly stressed the importance and need for stakeholder involvement at all stages of the risk-based decision-making process (NAS, 2009.) Prior to this, in 1998 the UK Royal Commission on Environmental Pollution published a report on Setting Environmental Standards (RCEP, 1998) in which it emphasised that decisions must be informed by an early understanding of peoples values, with a process that ensures transparency and openness. This is a view that is shared by the chemicals industry (Webb et al., 2003).

The Royal Commission also identified specific mechanisms by which this could be achieved. It

stated that those affected have a right to make their views known before a decision is made and that 'it is no longer acceptable for decisions to be negotiated privately between the regulator and polluter' (RCEP, 1998). This was fully endorsed by the UK government in their response to the Commission's report. But this endorsement is yet to translate into action. Opportunities for engagement and consultation that do exist are insufficient⁽¹⁴⁾.

Decisions regarding regulation of EE2 and its sister oestrogens, and the dilemmas and issues these pose, have been, and continue to be undertaken in a poorly understood, closed process that has little engaged the public. It is vitally important that decision-makers understand about the acceptability of risk, appetite for precaution and the willingness to pay for being precautionary. The views and concerns of the public have to date however gone largely undocumented. This is not an academic exercise: it is fundamental to making a decision on such a complex and potentially costly issue, a point emphasised by the Royal Commission in 1998 and the European Commission in 2000. Without public support the costs of risk management, of regulation of EE2, may be seen by policy makers as disproportionate. It might be that the weight of evidence suggests that public opinion is not on the side of risk management, that we are prepared to live with endocrine disruption in the environment as collateral damage associated with flexible fertility in our own species. But what is very clear is that without asking the public it will be far easier to come to a conclusion based largely on costs alone. This loads the dice before they are thrown.

⁽¹⁴⁾ For example in the development of the next round of River Basin Management Plans across the EU under the Water Framework Directive.

Table 13.1 Early warnings and actions

1938	17 α -ethinyl estradiol (EE2) synthesised
1943	EE2 marketed as a contraceptive
1962–1969	USFDA approve the birth control pill and the United Kingdom allows it to be prescribed through its National Health Service
1970	Pill users increase in number from 50 000 to 1 million
1976	First speculation that oral contraceptives might pass through sewage treatment works (STWs) into the aquatic environment
1979	Kinloch Nelson and Bunge publish a study showing low sperm counts in 93 % of men about to undergo vasectomies
1982	Routine health checks of male fish (roach) in a UK river show the presence of oocytes in testes (intersex). The rate of hermaphroditism is very high in comparison to the norm
Mid-1980s	First reports of contraceptive pill hormones in river water
1985	High levels of female-specific yolk protein (vitellogenin) found in blood of male fish in a fish farm receiving effluent containing river water
1985	Nonylphenols discovered in sewage effluents and in sludge
1991	National survey shows that oestrogenic effluents are widespread in England and Wales
1991	4-Nonylphenol is rediscovered as an oestrogen
1992	Meta-analysis of 61 studies shows sperm counts have declined 50 % in the preceding 50 years
1993	Theo Colborn and Clement publish 'The Wildlife Human Connection' suggesting widespread endocrine disrupting effects in wildlife and humans as a result of exposure to chemicals
1994–1996	Sharpe and Skakkebaek publish a hypothesis that testicular cancer, hypospadias, cryptorchidism and lowered semen quality are part of a syndrome caused by exposure to environmental oestrogens during foetal life
1993	BBC Horizon screens the award-winning documentary 'Assault on the male'
1995–1996	Surveys show that intersex is widespread in roach and is especially prevalent downstream of STWs with low effluent dilution. Feminising syndromes in other wildlife species are reported
1996	Major European workshop on endocrine disruptors are held, jointly sponsored by the European Commission, the European Environment Agency, the European Centre for Environment and Health and the World Health Organization
1998	Steroid oestrogens, and EE2 in particular, are shown to be the most potent oestrogenically active substances in domestic effluent
1998	Royal Commission on Environmental Pollution publishes a report <i>Setting environmental standards</i> , in which it emphasises that decisions must be informed by an early understanding of people's values. It is endorsed by the United Kingdom government
1999	European Union launches a Strategy on endocrine disrupters
2001	Feminisation is shown to be a permanent phenomenon that is progressive with age i.e. duration of exposure
2001	Schering publish a whole life-cycle study showing EE2 causing full sex reversal of males to females at concentrations > 2 ng/L
2002	Silva et al. show additive effects of oestrogenic endocrine disrupting chemicals <i>in vitro</i> : 'something from nothing'
2002–2003	More widespread surveys show intersex fish are present throughout the United Kingdom. Widespread anti-androgenic activity is discovered in sewage effluents
2003	Nonylphenol and nonylphenol ethoxylates are banned in the European Union as a hazard to human and environmental safety. Serious evaluations of other endocrine disruptors such as BPA take place
2004	Predicted No Effect Concentration (PNEC) of 0.1 ng/L derived for EE2
2004	UK Endocrine Disruption Demonstration Programme commences, evaluating the efficiency of removing oestrogens from sewage treatment processes
2007	Draft environmental quality standard for EE2 prepared by the Environment Agency of England and Wales
2008	Experimental lakes study in Canada reports a population crash of fish after exposure to EE2 at 6 ng/L
2009	UK Demonstration Programme reports first phase results showing difficulty removing EE2 to the PNEC using conventional sewage treatment approaches
2012	European Commission publishes proposals to regulate EE2 as a 'Priority Substance' under the EU Water Framework Directive, which if accepted may come into force after 2015. An amendment to this proposal is tabled in July 2012 which proposes delay of setting EQS until 2016, with the aim of meeting this by 2027'

References

- Adami, H.-O., Bergström, R., Möhner, M., Zatonski, W., Storm, H., Ekblom, A., Tretli, S., Teppo, L., Ziegler, H., Rahu, M., Gurevicius, R. and Stengrevics, A., 1994, 'Testicular cancer in nine Northern European countries', *Int. J. Cancer*, (59) 33–38.
- Aherne, G.W., English, J. and Marks, V., 1985, 'The role of immunoassay in the analysis of microcontaminants in water samples', *Ecotoxicol. Environ. Saf.*, (9) 79–83.
- Allen, Y., Scott, A.P., Matthiessen, P., Haworth, S., Thain, J.E. and Feist, S., 1999, 'Survey of estrogenic activity in United Kingdom estuarine and coastal waters and its effects on gonadal development of the flounder *Platichthys flesus*', *Environ. Toxicol. Chem.*, (18) 1 791–1 800.
- Apoteket AB, Stockholm County Council, Stockholm University, 2006, 'Environment and Pharmaceuticals', Apotek AB (The National Association of Swedish Pharmacies), Stockholm.
- Apoteket AB, MistraPharma and Stockholm County Council, 2010, 'A Health Future: pharmaceuticals in a Sustainable Society', Stockholm.
- Asbell, B., 1995, *The Pill: a biography of the drug that changed the world*, Random House, New York.
- Baynes, A., Green, C., Nicol, E., Beresford, N., Kanda, R., Henshaw, A., Churchley, J., and Jobling, S., 2012, 'Additional Treatment of Wastewater Reduces Endocrine Disruption in Wild Fish—A Comparative Study of Tertiary and Advanced Treatments', *Environmental Science & Technology*, (46/10) 5 565–5 573.
- Bergström, R., Adami, H.-O., Möhner, M., Zatonski, W., Storm, H., Ekblom, A., Tretli, S., Teppo, L., Akre, O. and Hakulinen, T., 1996, 'Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon', *J. Natl. Cancer Inst.*, (88) 727–733.
- Berthelsen, J.G., 1984, *Andrological aspects of testicular cancer*, Scriptor, Copenhagen.
- Bjerregaard, L.B., Korsgaard, B. and Bjerregaard, P., 2006, 'Intersex in wild roach (*Rutilus rutilus*) from Danish sewage effluent-receiving streams', *Ecotoxicol. Environ. Saf.*, (64) 321–328.
- Blazer, V.S., Iwanowicz, L.R., Iwanowicz, D.D., Smith, D.R., Young, J.A., Hedrick, J.D., Foster, S.W. and Reeser, S.J., 2007, 'Intersex (*testicular oocytes*) in Smallmouth Bass from the Potomac River and selected nearby drainages', *J. Aq. An. Health*, (19) 242–253.
- Brian, J.V., Harris, C.A., Scholze, M., Backhaus, T., Booy, P., Lamoree, M., Pojana, G., Jonkers, N., Runnalls, T., Bonfa, A., Marcomini, A. and Sumpter, J. P. 2005, 'Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals', *Environ. Health Perspect.*, (113) 721–728.
- Brian, J.V., Harris, C.A., Scholze, M., Kortenkamp, A., Booy, P., Lamoree, M. Pojana, G., Jonkers, N. Marcomini, A. and Sumpter, J.P., 2007, 'Evidence of estrogenic mixture effects on the reproductive performance of fish', *Environ. Sci. Technol.*, (41) 337–344.
- Caldwell, D.J., Mastrococo, F., Hutchinson, T.H., Lange, R., Heijerick, D., Janssen, C., Anderson, P.D. and Sumpter, J.P., 2008, 'Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17 α -ethinyl estradiol', *Environ. Sci. Technol.*, 42(7) 046–7 054.
- Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N.E., 1992, 'Evidence for decreasing quality of semen during past 50 years', *Brit. Med. J.*, (305) 609–613.
- CBC Digital Archives, 1964, *This Hour Has Seven Days* (http://archives.cbc.ca/health/reproductive_issues/topics/572). Birth control: the magic pill (http://archives.cbc.ca/health/reproductive_issues/clips/2956/).
- Cho, S.-M., Kurihara, R., Strussmann, C.A., Uozumi, M., Yamakawa, H., Yamasaki, T., Soyano, K., Shiraishi, H., Morita, M. and Hashimoto, S., 2003, 'Histological abnormalities in the gonads of konoshiro gizzard shad (*Konosirus punctatus*) from coastal areas of Japan' *Environ. Sci.*, (10/1) 25–36.
- Clement, C. and Colborn, T. (eds), 1992, *Chemically-induced alterations in sexual and functional development: the wildlife/human connection — Advances in modern toxicology volume XXI*, Princeton Scientific Publishing Inc., Princeton, NJ.
- Clemmesen, J., 1968, 'A doubling of morbidity from testis carcinoma in Copenhagen, 1943–1962', *APMIS* (72) 348–349.
- De Metrio, G., Corriero, A., Desantis, S., Zubani, D., Cirillo, F., Deflorio, M., Bridges, C.R., Eicker, J., de la Serna, J.M., Megalofonou, P. and Kime, D.E., 2003, 'Evidence of a high percentage of intersex in the

- Mediterranean swordfish (*Xiphias gladius* L.), *Mar. Pollut. Bull.*, (46) 358–361.
- DeQuattro, Z.A., Peissig, E.J., Antkiewicz, D.S., Lundgren, E.J., Hedman, C.J., Hemming, J.D. and Barry, T.P., 2012, 'Effects of progesterone on reproduction and embryonic development in the fathead minnow (*Pimephales promelas*)', *Environmental Toxicology and Chemistry*, doi:10.1002/etc.1754.
- Den Hond, E. and Schoeters, G., 2006, 'Endocrine disrupters and human puberty', *Int. J. Androl.*, (29) 264–271; 286–290.
- Desbrow, C., Routledge, E. J., Brighty, G.C., Sumpter, J.P. and Waldock, M., 1998, 'Identification of estrogenic chemicals in STW effluent, I: Chemical fractionation and in vitro biological screening', *Environ. Sci. Technol.*, (32) 1 549–1 558.
- Diniz, M.S., Peres, I., Magalhaes-Antoine, I., Falla, J. and Pihan, J.C., 2005, 'Estrogenic effects in crucian carp (*Carassius carassius*) exposed to treated sewage effluent', *Ecotoxicol. Environ. Saf.*, (62) 427–435.
- Dodds, E.C. and Lawson, W., 1935, 'Synthetic oestrogenic agents without the phenanthrene nucleus', *Nature*, (137/3476) 996.
- Dodds, E.C., and Lawson, W., 1938, 'Molecular structure in relation to oestrogenic activity — Compounds without a phenanthrene nucleus', *Proc. R. Soc. B.*, (125) 222–232.
- Dodds, E.C., Goldberg, L., Lawson, W. and Robinson, R., 1938, 'Oestrogenic activity of certain synthetic compounds', *Nature*, (141) 247–248.
- Eawag, 2009, 'Ozonung von gerreinigtem Abwasser', Eawag Aquatic Research.
- EC, 2000, Communication from the Commission on the precautionary principle, Brussels, 02.02.2000 COM(2000)1.
- EC, 2012, Proposal for a Directive of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Brussels, 31.1.2012 COM(2011) 876 final 2011/0429 (COD).
- EEA, 2001, *Late lessons from early warnings: the precautionary principle 1896–2000*, Environmental issues report No 22, European Environment Agency.
- EEA, 2010, *Pharmaceuticals in the Environment*, EEA Technical report No 1/2010, European Environment Agency.
- EEA, Academy of Finland, European Commission (DG Research), 2012, *The impacts of endocrine disrupters on wildlife, people and their environments — The Weybridge+15 (1996–2011) report*, edited by Sue Jobling and Jorma Topari, Technical report No 2/2012, European Environment Agency.
- EMA, 2006, *Guideline on the environmental risk assessment of medicinal products for human use*, EMEA/CHMP/SWP/4447/00, European Medicines Agency, London, the United Kingdom.
- EU, 1996, 'European Workshop on the impact of endocrine disrupters on human health and wildlife, 2–4 December 1996' (http://ec.europa.eu/environment/endocrine/documents/reports_en.htm) accessed 31 January 2012.
- EU, 1999, 'EU strategy on endocrine disruption' (http://ec.europa.eu/research/endocrine/activities_strategy_en.html) accessed 31 January 2012.
- Filby, A.L., Thorpe, K.L., Maack, G., Tyler, C. R., 2007, 'Gene expression profiles revealing the mechanisms of anti-androgen- and oestrogen- induced feminisation in fish', *Aquatic Toxicology*, (81/2) 219–231.
- Filby, A.L., Neuparth, T., Thorpe, K. L., Owen, R., Galloway, T.S., Tyler, C.R., 2007, 'Health impacts of estrogens in the environment, considering complex mixture effects', *Environmental Health Perspectives*, (115) 1 704–1 710.
- Filby, A.L., Shears, J.A., Drage, B.E., Churchley, J.H. and Tyler, C.R., 2010, 'Effects of advanced treatments of wastewater effluents on estrogenic and reproductive health impacts in fish', *Environ. Sci. Technol.*, (44/11) 4 348–4 354.
- Fisch, H., Goluboff, E.T., Olson, J.H., Feldshuh, J., Broder, S.J., Barad, D.H., 1996, 'Semen analyses in 1 283 men from the United States over a 25-year period: no decline in quality' *Fertil. Steril.*, (65) 1 009–1 014.
- Folmar, L.C., Denslow, N.D., Rao, V., Chow, M., Crain, A., Enblom, J., Marcino, J., Guillette Jr., L.J., 1996, 'Vitellogenin Induction and Reduced Serum Testosterone Concentrations in Feral Male Carp (*Cyprinus Carpio*) Captured Near a Major Metropolitan Sewage Treatment Plant', *Environ Health Perspect.*, (104) 1 096–1 100.

- Fossi, M.C., Casini, S., Marsili, L., Ancora, S., Mori, G., Neri, G., Romeo, T. and Ausili, A., 2004, 'Evaluation of eco-toxicological effects of endocrine disruptors during a four-year survey of the Mediterranean population of swordfish (*Xiphias gladius*)' *Mar. Environ. Res.*, (58) 425–429.
- Gee, D., 2006, 'Late lessons from early warnings: toward realism and precaution with endocrine disrupting substances', *Environmental Health Perspectives*, (114) 152–160.
- German Advisory Council on the Environment, 2007, *Pharmaceuticals in the Environment. Statement April 2007*, No. 12.
- Giger, W., Brunner, P.H., and Schaffner, C., 1984, '4-nonylphenol in sewage sludge; accumulation of toxic metabolites from nonionic surfactants', *Science*, (225) 623–625.
- Goldin, C. and Katz, L., 2002, 'The Power of the Pill: Oral Contraceptives and Women's Career and Marriage Decisions', *Journal of Political Economy*, (110/4) 730–770.
- Gross-Sorokin, M.Y., Roast, S.D., Brighty, G.C., 2006, 'Assessment of feminization of male fish in English rivers by the Environment Agency of England and Wales', *Environ. Health Perspect.*, (114/S-1) 147–151.
- Hannah, R., D'Aco, V.J., Anderson, P.D., Buzby, M.E., Caldwell, D.J., Cunningham, V.L., Ericson, J.F., Johnson, A.C., Parke, N.J., Samuelian, J.H. and Sumpter, J.P., 2009, 'Exposure assessment of 17 alpha ethinylestradiol in surface waters of the United States and Europe', *Environmental Toxicology and Chemistry*, (28/12) 2 725–2 732.
- Harris C.A., Hamilton P.B., Runnalls T.J., Jobling S., Vinciotti V., Henshaw A., Hodgson D., Coe T.S., Tyler C.R. and Sumpter J.P., 2011, 'The consequences of feminisation in breeding groups of wild fish', *Environmental Health Perspectives*, (119) 306–311.
- Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Routledge, E.J., Rycroft, R., Sumpter, J. P. and Tylor, T., 1996, 'A survey of estrogenic activity in United Kingdom inland waters', *Environmental Toxicology and Chemistry*, (15/11) 1 993–2 002.
- Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, M., Sumpter, J. P., Taylor, T. and Zaman, N., 1997, 'Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout', *Environmental Toxicology and Chemistry*, (16/3) 534–542.
- Hayashi, A., 2004, 'Japanese women shun the pill', CBS News (<http://www.cbsnews.com/stories/2004/08/20/health/main637523.shtml>) accessed 5 February 2012.
- Hayes, 2009, 'Birth control in Japan' (<http://factsanddetails.com/japan.php?itemid=599&catid=18>) accessed 5 February 2012.
- Hecker, M., Tyler, C.R., Maddix, S. and Karbe, L., 2002, 'Plasma biomarkers in fish provide evidence for endocrine modulation in the Elbe River, Germany', *Environ. Sci. Technol.*, (36/11) 2 311–2 321.
- Henley, D.V., Lipson, N., Korach, K.S. and Bloch, C.A., 2007, 'Prepubertal gynecomastia linked to lavender and tea tree oils', *Engl. J. Med.*, (356) 479–485.
- Higashitani, T., Tamamoto, H., Takahashi, A. and Tanaka, H., 2003, 'Study of estrogenic effects on carp (*Cyprinus carpio*) exposed to sewage treatment plant effluents', *Water Science and Technology*, (47) 93–100.
- Hinck, J.E., Blazer, V.S., Schmitt, C.J., Papoulias, D. M. and Tillitt, D. E., 2009, 'Widespread occurrence of intersex in black basses (*Micropetrus spp.*) from US rivers, 1995–2004', *Aquatic Toxicology*, (95) 60–70.
- Hohlbein, R., 1959, 'Mißbildungsfrequenz in Dresden', *Zentralblatt für Gynäkologie*, (18) 719–731.
- Hunter, G.A. and Donaldson, E.M., 1983, 'Hormonal sex control and its application to fish culture', *Fish Physiol.*, (9) 223– 303.
- Ibarreta, I. and Swan, S.H., 2002, 'The DES story: long-term consequences of prenatal exposure', in: *Late lessons from early warnings: the precautionary principle 1896–2000*, Environmental issue report No 22, European Environment Agency.
- Inhoffen, H.H. and Hohlweg, W., 1938, 'Neue per os-wirksame weibliche Keimdrüsenhormon-Derivate: 17-Aethinyl-oestradiol und Pregnen-in-on-3-ol-17', *Naturwissenschaften*, (26/6) 96.
- Jacobson-Dickman, E. and Lee, M.M., 2009, 'The influence of endocrine disruptors on pubertal timing', *Curr. Opin. Endocrinol. Diabetes Obes.*, (16/1) 25–30.
- Jafri, S.I.H. and Ensor, D.M., 1979, 'Occurrence of an intersex condition in the roach *Rutilus rutilus* (L)', *Journal of Fish Biology*, (14/6) 547–549.
- Jobling, S. and Sumpter, J.P., 1993, 'Detergent components in sewage effluent are weakly oestrogenic to fish: An in-vitro study using rainbow

- trout (*Oncorhynchus mykiss*) hepatocytes', *Aquatic Toxicology*, (27) 361–372.
- Jobling, S., Sheahan, D., Osborne, J.A., Matthiessen, P. and Sumpter, J.P., 1996, 'Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals', *Environmental Toxicology and Chemistry*, (15/2) 194–202.
- Jobling, S., Nolan, M., Tyler, C.R., Brighty, G. and Sumpter, J.P., 1998, 'Widespread sexual disruption in wild fish', *Environ. Sci. Technol.*, (32/17) 2 498–2 506.
- Jobling, S., Beresford, N., Nolan, M., Rodgers-Gray, R., Tyler, C.R., and Sumpter, J.P., 2002a, 'Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents', *Biol. Reprod.*, (66/2) 272–281.
- Jobling, S., Coey, S., Whitmore, J.G., Kime, D.E., Van Look, K.J.W., McAllister, B.G., Beresford, N., Henshaw, A.C., Brighty, G., Tyler, C.R. and Sumpter, J.P., 2002b, 'Wild intersex roach (*Rutilus rutilus*) have reduced fertility', *Biol. Reprod.*, (67) 515–524.
- Jobling, S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nolan, M., Tyler, C.R., van Aerle, R., Santos, E. and Brighty, G., 2006, 'Predicted exposures to steroid estrogens in UK rivers correlate with widespread sexual disruption in wild fish populations', *Environmental Health Perspectives*, (114/S1) 32–39.
- Jobling, S., Burn, R.W., Thorpe, K., Williams, R. and Tyler, C., 2009, 'Statistical Modelling suggests that anti-androgens from wastewater treatment works contribute to widespread sexual disruption in fish living in English rivers', *Environ. Health Perspect.*, (117) 797–802.
- Johnson, A.C., Yoshitani, J., Tanaka, H., Suzuki, Y., 2011, 'Predicting national exposure to a point source chemical: Japan and endocrine disruption as an example', *Environmental Science & Technology*, (45/3) 1 028–1 033
- Joffe, M., 2002, 'Myths about endocrine disruption and the male reproductive system should not be propagated', *Hum. Reprod.*, (17) 520–521.
- Jørgensen, N., Asklund, C., Carlsen, E. and Skakkebaek, N.E., 2006, 'Coordinated European investigations of semen quality: results from studies of Scandinavian young men is a matter of concern', *Int. J. Androl.*, (29/1) 54–61.
- Jouannet, P., Wang, C., Eustache, F., Jensen, T. K. and Auger, J., 2001, 'Semen quality and male reproductive health: the controversy about human sperm concentration decline', *APMIS*, (109) 48–61.
- Kanda, R. and Churchley, J., 2008, 'Removal of endocrine disrupting compounds during conventional wastewater treatment', *Environmental Technology*, (29) 315–323.
- Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M. and Flick, R.W., 2007, 'Collapse of a fish population after exposure to a synthetic estrogen', *PNAS*, (104) 8 897–8 901.
- Kinloch Nelson, C.M. and Bunge, R.G., 1974, 'Semen analysis: evidence for changing parameters of male fertility potential', *The American Fertility Society*, (25) 503–507.
- Kirby, M.F., Allen, R.Y., Dyer, R.A., Feist, S.W., Katsiadaki, I., Matthiessen, P., Scott, A.P., Smith, A., Stentiford, G.D., Thain, J.E., Thomas, K.V., Tolhurst, L. and Waldock, M.J., 2004, 'Surveys of plasma vitellogenin and intersex in male flounder (*Platichthys flesus*) as measures of endocrine disruption by estrogenic contamination in United Kingdom estuaries: temporal trends, 1996 to 2001', *Environ. Toxicol. Chem.*, (23) 748–758.
- Kümmerer K, (ed.) 2004, *Pharmaceuticals in the environment: sources, fate, effects and risks*. Springer, 2nd ed., Berlin Heidelberg New York.
- Kümmerer K, 2007, *Sustainable from the very beginning: rational design of molecules by life cycle engineering as an important approach for green pharmacy and green chemistry*, *Green Chem* 9, pp. 899–907.
- Kuster, M., de Alda, M.J., Hernando, M.D., Petrovica, M., Martín-Alonsod, J. and Barceló, D., 2008, 'Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain)', *Journal of Hydrology*, (358/1–2) 112–123.
- Lakha, F., Ho, P.C., Van der Spuy, Z.M., Dada, K., Elton, R., Glasier, A. F., Critchley, H.O.D., Williams, A.R.W. and Baird, D.T., 2007, 'A novel estrogen-free oral contraceptive pill for women: multicentre, double-blind, randomized controlled trial of mifepristone and progestogen-only pill (levonorgestrel)' *Hum. Reprod.*, (22) 2 428–2 436.

- Lange, R., Hutchinson, T.H., Croudace, C.P., Siegmund, F., Schweinfurth, H., Hampe, P., Panter, G.H. and Sumpter, J.P., 2001, 'Effects of the synthetic oestrogen 17 alpha-ethinylestradiol over the life-cycle of the fathead minnow (*Pimephales promelas*)', *Environ. Toxicol. Chem.*, (20/6) 1 216–1 227.
- Lange, A., Katsu, Y., Ichikawa, R., Chidgey, L., Iguchi, T. and Tyler, C.R., 2009, 'Sexual reprogramming and oestrogen sensitisation in wild fish exposed to ethinylloestradiol', *Environmental Science and Technology*, (43/4) 1 219–1 225.
- Larsson, D.G.J., Adolfsson, E.M., Parkkonen, J., Pettersson, M., Berg, A. H., Olsson, P.E. and Forlin, L., 1999, 'Ethinylloestradiol – an undesired fish contraceptive?', *Aquat. Toxicol.*, (45) 91–97.
- Leridon, H., 2006, 'Demographic effects of the introduction of steroid contraception in developed countries', *Human Reproduction Update*, (12/5) 603–616.
- Liney, K.E., Hagger, J.A., Tyler, C.R., Depledge, M. H., Galloway, T.S. and Jobling, S., 2006, 'Health effects in fish after long-term exposure to effluents from wastewater treatment works', *Environmental Health Perspectives*, (114/1) 81–89.
- Lokke, 2006, 'The precautionary principle and chemicals regulation: past achievements and future possibilities', *Environ. Sci. Poll. Res.*, (13/5) 342–349.
- Lund, L., Engebjerg, M.C., Pedersen, L., Ehrenstein, V., Nørgaard, M., Sørensen, H.T., 2009, 'Prevalence of hypospadias in Danish boys: a longitudinal study, 1977–2005', *Eur Urol.*, (55/5) 1 022–1 026.
- Lye, C. M., Frid, C.L.J., Gill, M.E., McCormick, D., 1997, 'Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works', *Mar. Pollut. Bull.*, (34) 34–41.
- Lye, C.M., Frid, C.L.J. and Gill, M.E., 1998, 'Seasonal reproductive health of flounder *Platichthys flesus* exposed to sewage effluent', *Mar. Ecol. Prog. Ser.*, (170) 249–260.
- Lyons, G., 2008, *Effects of pollutants on the reproductive health of male vertebrate wildlife: males under threat*, CHEM Trust Report.
- Ma, T.W., Wan, X.Q., Huang, Q.H., Wang, Z.J. and Liu, J.K., 2005, 'Biomarker responses and reproductive toxicity of the effluent from a Chinese large sewage treatment plant in Japanese medaka (*Oryzias latipes*)', *Chemosphere*, (59) 281–288.
- MacLeod, J. and Heim, L.M., 1945, 'Characteristics and variations in semen specimens in 100 normal young men', *J. Urol.*, (54) 474–482.
- MacLeod, J. and Wang, Y., 1979, 'Male fertility potential in terms of semen quality: a review of the past, a study of the present', *Fertil. Steril.*, (3) 103–116.
- Margel, D. and Fleshner, N.E., 2011, 'Oral contraceptive use is associated with prostate cancer: an ecological study', *BMJ*, (1) e000311, doi:10.1136/bmjopen-2011-000311.
- Martin, O.V., Shialis, T., Lester, J.N., Scrimshaw, M. D., Boobis, A.R. and Voulvoulis, N., 2008, 'Testicular dysgenesis syndrome and the estrogen hypothesis: a quantitative meta-analysis', *Environ. Health Perspect.*, (116) 149–157.
- Medical News, 1961, 'Contraceptive Pill', *Br. Med. J.*, (2/5266) 1 584.
- Maisel, A.Q., 1965, *The hormone quest*, Random House, New York.
- Matlai, P. and Beral, V., 1985, 'Trends in congenital malformations of external genitalia', *The Lancet*, (i) 108.
- McGlynn, K.A., Devesa, S.S., Sigurdson, A.J., Brown, L.M., Tsao, L. and Tarone, R.E., 2003, 'Trends in the incidence of testicular germ cell tumors in the United States', *Cancer*, (97/1) 63–70.
- McLachlan, J.A., 1980, *Estrogens in the environment – Developments in toxicology and environmental science, Volume 5*, Elsevier, Holland.
- Mistrapharma, 2011, Newsletter of the Mistrapharma research project, contact: Christina at cr@abe.kth.se.
- Moller, H., 2001, 'Trends in incidence of testicular cancer and prostate cancer in Denmark', *Hum. Reprod.*, (16/5) 1 007–1 011.
- Nash, J.P., Kime, D.E., van der Ven, L.T.M., Wester, P.W., Brion, F., Maack, G., Stahlschmidt-Allner, P. and Tyler C.R., 2004, 'Environmental concentrations of the pharmaceutical ethinylloestradiol cause reproductive failure in fish', *Environmental Health Perspectives*, (112) 1 725–1 733.
- NAS, 2009, *Science and decision making: advancing risk assessment*, National Academy of Sciences.
- Nature, 2011, 'Reduce drug waste in the environment', Letters vol. 478, 6 October, Michael

- Depledge, European Centre for Environment and Human Health, University of Exeter, the United Kingdom.
- NDP, 2010, *Assessment of the performance of wastewater treatment works in removing oestrogenic substances*, National Demonstration Programme.
- Ohkubo, N., Mochida, K., Adachi, S., Hara, A., Hotta, K., Nakamura, Y. and Matsubara, T., 2003, 'Estrogenic activity in coastal areas around Japan evaluated by measuring male serum vitellogenins in Japanese common goby *Acanthogobius flavimanus*', *Fish Sci.*, (69) 1 135–1 145.
- Owen, R. and Jobling, S., 2012, 'The hidden costs of flexible fertility', *Nature*, (485) 441.
- Paulsen, C.A., Berman, N.G. and Wang, C., 1996, 'Data from men in greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform', *Fertil. Steril.*, (65) 1 015–1 020.
- Paulos, P., Runnalls, T.J., Nallani, G., La Point, T., Scott, A.P., Sumpter, J.P., Huggett, D.B., 2010, 'Reproductive responses in fathead minnow and Japanese medaka following exposure to a synthetic progestin', *Norethindrone. Aquatic Toxicology*, (99/2) 256–262.
- Paulozzi, L.J., Erickson, J.D. and Jackson, R.J., 1997, 'Hypospadias trends in two US surveillance systems', *Pediatrics*, (100/5) 831–834.
- Penaz, M., Svobodova, Z., Barus, V., Prokes, M. and Drastichova, J., 2005, 'Endocrine disruption in a barbell, *Barbus barbus* population from the River Jihlava, Czech Republic', *J. Appl. Ichthyol.*, (21) 420–428.
- Petersen, P.M., Giwercman, A., Hansen, S.W., Berthelsen, J.G., Daugaard, G., Rorth, M. and Skakkebaek, N.E., 1999, 'Impaired testicular function in patients with carcinoma-in-situ of the testis', *J. Clin. Oncol.*, (17/1) 173–179.
- Prener, A., 1992, *Epidemiology — risk factors for testicular cancer with special focus on childhood exposure*, Danish Cancer Registry, Copenhagen.
- Purdom, C.E., Hardiman, P.A., Bye, V.V.J., Eno, N.C., Tyler, C.R. and Sumpter, J.P., 1994, 'Estrogenic effects of effluents from sewage treatment works', *Chemistry and Ecology*, (8) 275–285.
- Racz, L. and Goel, R.K., 2010, 'Fate and removal of estrogens in municipal wastewater', *J. Environ. Monit.*, (12) 58–70.
- Raman-Wilms, L., Tseng, A.L., Wighardt, S., Einarson, T.R. and Koren, G., 1995, 'Fetal genital effects of first trimester sex hormone exposure: a meta-analysis', *Obstet. Gynecol.*, (85) 141–149.
- RCEP, 1998, *Royal Commission on Environmental Pollution Report no 21: Setting Environmental Standards*.
- Richiardi, L., Bellocco, R., Adami, H.O., Torrang, A., Barlow, L., Hakulinen, T., Rahu, M., Stengrevics, A., Storm, H., Tretli, S., Kurtinaitis, J., Tyczynski, J.E. and Akre, O., 2004, 'Testicular cancer incidence in eight northern European countries: secular and recent trends', *Cancer Epidemiol. Biomarkers Prev.*, (13/12) 2 157–2 166.
- Rodgers-Gray, T.P., Jobling, S., Janbakash, A., Sumpter, J.P. and Tyler, C.R., 2001, 'Exposure of roach to sewage effluents during sexual differentiation causes permanent feminisation of the reproductive ducts', *Environ. Sci. and Tech.*, (35) 462–470.
- Routledge, E.J., Sheahan, D., Desbrow, C., Brighty, G.C., Waldock, M. and Sumpter, J.P., 1998, 'Identification of estrogenic chemicals in STW effluent. II : In vivo responses in trout and roach', *Environ. Sci. Technol.*, (32) 1 559–1 565.
- Santillo, S., Johnston, P. and Langston, W.J., 2001, 'Tributyltin (TBT) antifoulants: a tale of ships, snails and imposex', in: *Late lessons from early warnings: the precautionary principle 1896–2000*, Environmental issue report No 22, European Environment Agency.
- Schnack, T.H., Poulsen, G., Myrup, C., Wohlfahrt, J., Melbye, M., 2009, *Familial coaggregation of cryptorchidism, hypospadias, and testicular germ cell cancer: a nationwide cohort study*, Natl Cancer Inst.
- Science Blog, 2004, *UK to tackle endocrine disruptors in wastewater*.
- Scott, A.P., Katsiadaki, I., Witthames, P.R., Hylland, K., Davies, I.M., Macintosh, A.P. and Thain, J., 2006, 'Vitellogenin in the blood plasma of male cod (*Gadus morhua*): a sign of oestrogenic disruption in the open sea?', *Marine Environ. Res.*, (61) 149–170.
- Scott, A.P., Sanders, M., Stentiford, G.D., Reese, R.A. and Katsiadaki, I., 2007, 'Evidence for estrogenic endocrine disruption in and offshore flatfish, the dab (*Limanda limanda* L.)', *Marine Environ. Res.*, (64) 128–148.

- Sharpe, R.M., 2009, *Male reproductive health disorders and the potential role of exposure to environmental chemicals*, Chem. Trust Report.
- Sharpe, R.M. and Skakkebaek, N.E., 1993, 'Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract?', *The Lancet*, (341) 1 392–1 395.
- Sheahan, D.A., Bucke, D., Matthiessen, P., Sumpter, J.P., Kirby, M.G., Neall, P. and Waldock, M., 1994, 'The effects of low levels of 17 α -ethynylestradiol upon plasma vitellogenin levels in male and female rainbow trout, *Oncorhynchus mykiss* (Walbaum), held at two acclimation temperatures', in: Miller, R. and Lloyd, R. (eds), *Sublethal and chronic effects of pollutants on freshwater fish*, Fishing News Books, Oxford.
- Sheahan, D.A., Brighty, G.C., Daniel, M., Jobling, S., Harries, J.E., Hurst, M.R., Kennedy, J., Kirby, S.J., Morris, S., Routledge, E.J., Sumpter, J.P. and Waldock, M.J., 2002a, 'Reduction in the estrogenic activity of a treated sewage effluent discharge to an English river as a result of a decrease in the concentration of industrially derived surfactants', *Environ. Toxicol. and Chem.*, (21/3) 515–519.
- Sheahan, D.A., Brighty, G.C., Daniel, M., Kirby, S., Hurst, M.R., Kennedy, J., Morris, S., Routledge, E.J., Sumpter, J.P. and Waldock, M.J., 2002b, 'Estrogenic activity measured in a sewage treatment works treating industrial inputs containing high concentrations of alkylphenolic compounds a case study', *Environ. Toxicol. Chem.*, (21) 507–514.
- Silva, E., Rajapakse, N. and Kortenkamp, A., 2002, 'Something from "nothing" — Eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects', *Environ. Sci. Technol.*, (36/8) 1 751–1 756.
- Skakkebaek, N.E., Rajpert-De Meyts, E., Jorgensen, N., Carlsen, E., Petersen, P.M., Giwercman, A., Andersen, A.G., Jensen, T.K., Andersson, A.M. and Müller, J., 1998, 'Germ cell cancer and disorders of spermatogenesis: an environmental connection?', *APMIS*, (106) 3–11.
- Skakkebaek, N.E., 2010, 'Normal reference ranges for semen quality and their relations to fecundity', *Asian Journal of Andrology*, (12) 95–98.
- Smithells, R.W., 1981, 'Oral contraceptives and birth defects', *Develop. Med. Child Neurol.*, (23) 369–383.
- Sneader, W., 1985, *Drug discovery: the evolution of modern medicines*, Wiley.
- Snyder, S.A., Villeneuve, D.L., Snyder, E.M. and Giesy, J.P., 2001, 'Identification and quantification of estrogen receptor agonists in wastewater effluents', *Environ. Sci. Technol.*, (35) 3 620–3 625.
- Soto, A.M., Justicia, H., Wray, J.W. and Sonnenschein, C., 1991, 'P-nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene', *Environ. Health Perspect.*, (92) 167–173.
- Standley, L.J., Rudel, R.A., Swartz, C.H., Attfield, K.R., Christian, J., Erickson, M. and Brody, J.G., 2008, 'Wastewater-contaminated groundwater as a source of endogenous hormones and pharmaceuticals to surface water ecosystems', *Environmental Toxicology and Chemistry*, (27/12) 2 457–2 468.
- Sumpter, J.P. and Jobling, S., 1995, 'Vitellogenesis as a biomarker of oestrogenic contamination of the aquatic environment', *Environ. Health. Persp.*, (103/7) 173–178.
- Sumpter, J.P. and Johnson, A.C., 2008, '10th anniversary perspective: reflections on endocrine disruption in the aquatic environment: from known knowns to unknown unknowns (and many things in between)', *J. Environ. Monit.*, (10) 1 476–1 485.
- Swan, S.H., Elkin, E.P. and Fenster, L., 1997, 'Have sperm densities declined? A reanalysis of global trend data', *Environ. Health Perspect.*, (105) 1 228–1 232.
- Sweet, R.A., Schrott, H.G., Kurland, R. and Culp, O.S., 1974, 'Study of the incidence of hypospadias in Rochester, Minnesota, 1940–1970, and a case-control comparison of possible etiologic factors', *Mayo Clin. Proc.*, (49) 52–58.
- Sweeting, R. A., 1981, *Report to Thames Water*.
- Tabak, H.H., and Bunch, R.L., 1970, 'Steroid hormones as water pollutants', in: Society for Industrial Microbiology (ed.) *Developments in Industrial Biology*. Volume 11 *Proceedings of the Twenty-Sixth General Meeting of the Society for Industrial Microbiology Held in Burlington, Vermont, August 17–22, 1969*, Am. Inst. Biol. Sci., Washington DC.
- Tabak, H.H., Bloomhuff, R.N. and Bunch, R.L., 1981, 'Steroid hormones as water pollutants, II Studies on the persistence and stability of natural urinary and synthetic ovulation-inhibiting hormones in untreated and treated wastewaters', *Dev. Ind. Microbiol.*, (22) 497–519.
- Tanaka, H., Yakou, Y., Takahashi, A., Komori, K., Okayasu, Y., 2001, In Source: Proceedings of the Water Environment Federation, WEFTEC 2001;

- Water Environment Federation: Washington DC, pp. 632–651.
- Taylor, A.A.L., Jobling, S., Nolan, M., Tyler, C.R., van Aerle, R. and Brighty, G., 2005, *Spatial Survey of the Extent of Sexual Disruption in Wild Roach in English and Welsh Rivers*, Environment Agency Science Report P6-018/SR.
- Taylor, T., Keyse, L., and Bryant, A., 2006, *Contraception and Sexual Health*, 2005/06, Office for National Statistics, London.
- Time, 1961, 'Subsidizing birth control', *Time*, (78/24).
- Time, 1967, 'The Pill', *Time*.
- Thorpe, K.L., Cummings, R.I., Hutchinson, T.H., Scholze, M., Brighty, G., Sumpter, J.P., Tyler, C.R., 2003, 'Relative potencies and combination effects of steroidal estrogens in fish', *Environ. Sci. Technol.*, (37) 1 142–1 149.
- Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette, L.J. Jr, Jégou, B., Jensen, T.K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J.A., Meyer, O., Müller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J. and Skakkebaek, N.E., 1996, 'Male reproductive health and environmental xenoestrogens', *Environ. Health Perspect.*, (104) 162–163.
- Tyler, C.R., Jobling, S. and Sumpter, J.P., 1998, 'Endocrine disruption in wildlife: A critical review of the evidence', *Critical Reviews in Toxicology*, (28/4) 319–361.
- Tyler, C.R., Filby, A.L., Hill, E.M., Liney, K.E., Katsu, Y., Bickley, L.K., Lange, A., Winter, M., Gibson, R., Shears, J.A., Iguchi, T., 2009, 'Environmental health impacts of equine estrogens derived from hormone replacement therapy (HRT)', *Environmental Science and Technology*.
- Tyler, C.R. and Jobling, S., 2008, 'Fish, sex and gender-bending chemicals — the feminization of fish living in English rivers', *Biosciences*.
- UKWIR, 2009, *Endocrine disrupting chemicals national demonstration programme. Assessment of the performance of WwTW in removing oestrogenic substances*, Report ref 09/TX/04/16.
- UN, 1992, *Precautionary principle — Principle 15 of the UN Rio Declaration on Environment and Development*.
- UNPD, 2006, *World contraceptive use 2005*, United Nations Population Division, New York.
- Vajda, A.M., Barber, L.B., Gray, J.L., Lopez, E. M., Woodling, J.D. and Norris, D.O., 2008, 'Reproductive disruption in fish downstream from an estrogenic wastewater effluent', *Environ Sci Technol.*, (42/9) 3 407–3 414.
- Vermeirssen, E.L.M., Burki, R., Joris, C., Peter, A., Segner, H., Suter, M.J.F. and Burkhardt-Holm, P., 2005, 'Characterization of the estrogenicity of Swiss midland rivers using a recombinant yeast bioassay and plasma vitellogenin concentrations in feral male brown trout', *Environ. Toxicol. Chem.*, (24) 2 226–2 233.
- Vethaak, A.D., Lahr, J., Schrap, S. M., Belfroid, A.C., Rijs, G.B.J., Gerritsen, A., de Boer, J., Bulder, A.S., Grinwis, G.C., Kuiper, R.V., Legler, J., Murk, T.A., Peijnenburg, W., Verhaar, H.J. and de Voogt, P., 2005, 'An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of the Netherlands', *Chemosphere*, (59) 511–524.
- Wanderas, E.H., Tretli, S., Fossa, S.D., 1995, 'Trends in incidence of testicular cancer in Norway 1955–1992', *Eur. J. Cancer*, (31A/12) 2 044–2 048.
- Webb, S., Taalman, R., Becker, R., Onuma, K., Igarashi, I., 2003, 'Risk perception: A chemical industry view of endocrine disruption in wildlife', *Pure Appl. Chem.*, (75) 2 575–2 591.
- Weiner, J.B., Rogers, M.D., 2002, 'Comparing precaution in the United States and Europe', *Journal of Risk Research*, (5/4) 317–349.
- White, R., Jobling, S., Hoare, S.A., Sumpter, J.P. and Parker, M.G., 1994, 'Environmentally persistent alkylphenolic compounds are estrogenic', *Endocrinology*, (135/1) 175–182.
- WHO, 2002, *Global assessment of the state-of-the science of endocrine disruptors*, an assessment prepared by an expert group on behalf of the World Health Organization, the International Labour Organisation, and the United Nations Environment Programme.
- WHO, 1991, *Congenital malformations worldwide: a report from the international clearinghouse for birth defects monitoring systems*, Elsevier, Oxford.
- WHO, 1980, *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical*

Mucus Interaction, World Health Organization, Press Concern, Singapore.

Williams, R.J., Johnson, A.C., Jennifer, J., Smith, L. and Kanda, R., 2003, *Steroid estrogens profiles along river stretches arising from sewage treatment works discharges*.

Williams, R., Johnson, A., Keller, V., Young, A., Holmes, M., Wells, C., 2008, *Catchment risk assessment*

of steroid oestrogens from sewage treatment works, CEH project number C02996, science report SC030275/SR3, Environment Agency, Bristol.

Young, W.F., Whitehouse, P., Johnson, I. and Sorokin, N., 2004, *Proposed Predicted-No-Effect- Concentrations (PNECs) for natural and synthetic steroid oestrogens in surface waters*, EA R&D Technical Report P2-T04/1, Environment Agency, Bristol.