Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

COT statement on adverse trends in development of the male reproductive system – potential chemical causes

Introduction

1. In February 2003, the COT considered a number of possible discussion topics as part of its annual horizon scanning exercise. One of the topics considered was whether the Committee should contribute to ongoing debate of reported adverse trends in development of the male reproductive system and the possibility of chemicals being responsible for these reported effects. This statement summarises information reviewed by the Committee over a number of subsequent discussions.

2. Over the past 10-12 years an increasing body of literature has suggested there may have been a deterioration in human male reproductive health [1, 2, 3]. Examples of reported changes include a decline in sperm counts and other aspects of semen quality such as sperm morphology and motility, in some places but not others, [4, 5, 6 7,8, 9,10] a widespread and marked increased incidence of testicular cancer [11], and possible increases in hypospadias[12,13] and cryptorchidism[14]. Cryptorchidism is the most common developmental abnormality occurring in 2.2- 3.8% boys at birth [15], whereas the prevalence of hypospadias is between 1 in 125 live births and 1 in 250 new borns (0.4-0.8%)[16], and testicular cancer ranges from 0.0025% - 0.0085% [17]. Geographical and ethnic differences have also been reported in these conditions [3,11, 18, 19, 20, 21]. One consistent finding is that the reproductive health of Finnish men tends to be healthier than that of Danish or British men [3]. For instance using time to pregnancy as an indicator, Britain is reported to have lower fertility than Finland[22]. It is now recognised that some of the most important disorders of human male reproductive health may be caused or influenced by events in perinatal life. In addition over the last few decades, a decline in male to female sex ratios has been reported in Denmark, the Netherlands, England, Wales, the USA and Canada [23, 24,25].

3. Potential chemical causes of cancer fall within the remit of the Committee on Carcinogenicity (COC). However, the existence of parallel findings for the risk of testicular cancer with that of impaired semen quality, hypospadias and cryptorchidism, e.g. in Finland compared with Denmark, suggest linkage between these endpoints[3], and therefore testicular cancer was included in the COT consideration.

4. One hypothesis that has attracted much attention in recent years is that these observations on male reproductive health could be due to the effects of natural and synthetic endocrine disrupting chemicals (EDCs). The literature has been discussed extensively at meetings worldwide and in reports, including those of the Danish Environmental Protection Agency [26], the EU Weybridge workshop [27], the Royal Society [28], an expert panel of the International Programme on Chemical Safety (IPCS) [29], and an International Symposium convened by IUPAC/SCOPE [30]

5. Most initial reports of possible adverse effects of EDCs focused on effects in wildlife. The IPCS [29] report states that there is "strong evidence that there are effects observed in wildlife that can be attributed to substances that function as EDCs". Some examples are briefly highlighted below (paragraphs 9-12).

6. While a range of chemicals (both natural and man-made) have been shown to possess the potential to interfere with endocrine systems in experimental systems, it is now recognised that in most cases their potency and level of exposure is too low to have had any effect in humans, especially in the case of "environmental oestrogens" such as alkylphenolpolyethoxylates and bisphenol A [31, 32]. The potent oestrogen diethylstilboestrol (DES) has been recognised as an endocrine disrupting chemical with established effects on male reproductive development in humans, and this occurred only when it was given at pharmacological doses of more that 2000μg/kg/day during early pregnancy; however no effect was reported at lower doses [32].

7. Endocrine disruptors other than oestrogens could affect the male reproductive system, in particular those that inhibit the synthesis or the action of testosterone, such as phthalates or DDE (the stable breakdown product of DDT), respectively [31.] In addition they may act as anti-androgens to affect the androgen-oestrogen balance; studies in male rats have suggested the induction of reproductive tract abnormalities from the alteration of the androgen-oestrogen balance rather than from the absolute level of exposure to androgens or oestrogens[31]. Compounds may also interfere with the biosynthesis of oestrogen by suppressing the activity of aromatase, required for the conversion of testosterone to oestrogen. This may result in an excess of testosterone and hence could disturb the androgen-oestrogen balance. Such alterations to the production or action of oestrogen may lead to abnormal development of Sertoli and germ cells in the fetal testis, as these are sites containing oestrogen receptors [31].

8. However, the beginning of the rise in testicular cancer incidence predates the introduction of such chemicals, and the widespread use of DDT in malaria control programmes has not resulted in an epidemic of this disease[3]. Another possibility requiring further evaluation is whether exposure to dioxins, and/or to other substances that have a similar activity, could have adversely affected the male reproductive system. [33], However, as dietary exposure to dioxins has been decreasing for over 20 years [34] it is not likely to be responsible for the postulated increase in congenital abnormalities such as cryptorchidism and hypospadias.

Wildlife

9. It has been suggested that the abnormally small gonads and significantly depressed testosterone concentrations observed in juvenile male alligators from Lake Apopka, Florida could have been caused by contamination of the lake with various chemicals including the pesticide DDT and its metabolite DDE [35], [36]. This effect may explain the decline in the number of juvenile alligators in this area, whereas alligator populations elsewhere were increasing or stable at the same time.

10. Begeron *et al*.,[37] [38] demonstrated that a number of polychlorinated biphenyl (PCB) metabolites are capable of acting as synthetic estrogens. Some reptiles such as crocodilians and turtles exhibit environmental sex determination so that the temperature at which the egg is incubated determines the sex of the offspring [39]. Turtle eggs incubated at 26°C produce 100% males. However, if eggs incubated at the male producing temperature were "painted" with either one of the PCB metabolites (2',4',6'-trichloro-4-biphenylol or 2',3',4',5'-tetrachloro-4-biphenylol) sex reversal occurred as if the eggs had been treated with the natural estrogen, estradiol-17β [37].

11. The observation of an increased prevalence of hermaphroditism in fish from sewage treatment water (STW) lagoons in England and Wales initiated a series of studies examining the effects of environmental estrogens in STW-exposed rainbow trout. This was done using an assay measuring vitellogenin production. Vitellogenin produced in the liver of female fish stimulates the growth of ova and is under estrogenic control. In contrast, males do not produce vitellogenin unless exposed to supra-physiological levels of estrogens [40] which, can therefore be used as a biomarker for environmental estrogenic activity. STW-exposed caged rainbow trout showed an induction of 500-100,000-fold increase in plasma vitellogenin concentration and males were shown to achieve levels almost as high as females, indicating the contamination of water by estrogenic compounds [41]. These estrogenic compounds will include hormones excreted by women taking the contraceptive pill. A variety of adverse environmental conditions, including sub-optimal temperatures, restricted food supply, low pH, environmental pollutants and/or parasites, may also induce intersex effects in fish[42].

12. To date, the tributyltin (TBT)-induced masculinisation (imposex/intersex) in female molluscs, particularly prosobranch snails, is the best example of endocrine disruption in invertebrates that is causally linked to an environmental pollutant [43]. TBT is the active ingredient in antifouling paint applied below the waterline on ship hulls to prevent marine creatures from sticking to them. This application explains the wide-spread contamination of this compound in both fresh and seawater. TBT boosts the production of testosterone in female molluscs resulting in imposex, which is the growth of a penis and occlusion of the oviduct due to the development of a superficial vas deferens. The oviduct is blocked by the penis leading to sterility as reproduction is prevented. This is a key example of population-level impact resulting in a decline in population. TBT-containing antifouling paints have

been banned from use on vessels under 25 metres in length in the late 1980s, and recently the International Maritime Organisation called for a ban on any new application of TBT, with a total ban on the use of TBT by January 2008.

Experimental work

13. A large body of experimental work on the adverse effects of endocrine disrupting chemicals (EDCs) on male reproductive toxicity has been carried out both *in vitro*[44] and *in vivo* [45, 46, 47, 48]. However, the relevance of the data to the observed trends in human reproduction remain unclear.

14. The Organisation for Economic Co-operation and Development (OECD) began the development of new revised guidelines for the screening and testing of potential endocrine disrupters in 1998. One of these activities is validation of the rodent uterotrophic bioassay, an *in vivo* screen to identify suspected estrogen agonists or antoagonists of estrogen. The phase two validation studies are now complete. The OECD validation studies demonstrated that all four uterotrophic bioassay protocols were robust and reliable for identifying estrogen agonists and antagonists, and are transferable across laboratories [49]. The results from the validation studies have been submitted for independent peer-review to provide support for the validation of the uterotrophic bioassay, the data will be used to develop the draft OECD test guidelines for the uterotrophic bioassay.

15. The second assay under OECD validation is the *in vivo* Hershberger assay, which is based on the principle that the sex accessory tissues are under the control of androgens to stimulate and maintain growth. In the castrated male rat, sex accessory tissue weights can be restored by androgens, and similarly anti-androgens can block such effects. Phase 2 validation of this assay has also been completed [50,51,52]. All five accessory sex organs and tissues tested, consistently responded with statistically significant changes in weight, supporting the conclusion that the Hershberger assay is a reliable and reproducible assay for the detection of androgen agonistic and antagonistic effects. Phase 3 validation of the Hershberger assay will now follow.

16. Tinwell and Ashby have demonstrated that a variety of different estrogen receptor (ER) agonists, present individually at doses which are too low for an effect to be detected, can act simultaneously to evoke a ER-regulated response in the immature rat uterotrophic assay [53]. However simple addition of the activities overestimated the actual effect.

Recent and current activities on observations in humans

17. A current UK government research programme investigating the trends in male reproductive health and the possible effects of chemicals is in progress. This programme includes the following projects;

- An assessment and analysis of existing data on hypospadias in UK and Europe, publication of some of the results can be accessed at http://www.sickkids.on.ca/frontiersinfetalhealth/FFH_SeptOctNov2000.pdf

- Environmental risk factors for hypospadias: a population-based case control study in three health regions,

- Historically prospective cohort study of Scottish male reproductive health, additional information about this project is available on http://www.med.ed.ac.uk/hew/repro

- UK multi-centre study of occupational and environmental exposure to chemicals and male fertility.

18. A number of similar projects are in progress in the European Union, including:

- Inuendo - Biopersistent organochlorines in diet and human fertility. Epidemiological studies of time to pregnancy and semen quality in Inuit and European populations - further information is available at http://www.inuendo.dk.
- Envir.Reprod.Health - Increasing incidence of human male reproductive health disorders in relation to environmental effects on growth and sex steroid-induced alterations in programmed development
- EDEN - Endocrine disrupters: Exploring Novel Endpoints, Exposure, Low-Dose and Mixture-Effects in Humans, Aquatic Wildlife and Laboratory Animals - further information is available at http://www.credocluster.info/eden.html
- COMPRENDO - Comparative Research on Endocrine Disrupters, Phylogenetic Approach and Common Principles focussing on Androgenic/Antiandrogenic Compounds - further information is available at http://www.credocluster.info/comprendo.html
  Eurisked - Multi-organic risk assessment of selected endocrine disrupters – further information is available at http://www.credocluster.info/eurisked.html
- FIRE - Risk assessment of brominated flame retardants as suspected endocrine disrupters for human and wildlife health - further information is available at http://www.credocluster.info/fire.html
- CASCADE – Chemicals as contaminants in the food chain – further information is available at http://europa.eu.int/comm/research/endocrine/index_en.html

19. The potential adverse effects of phytoestrogens on the development of the male reproductive system were addressed by the COT Working Group on phytoestrogens; the report was published in May 2003 [54]. Studies on the effects of phytoestrogens on human reproductive development are limited in number, and there are no published human studies specifically investigating the potential effects of *in utero* exposure to phytoestrogens.

20. There are constraints on the ability to identify possible causes of adverse effects in the development of the human male reproductive system. This is due to the limitations that are common to epidemiological studies, as well as some specific problems such as uncertain ascertainment of hypospadias and cryptorchidism [21], and variations in population recruitment and in laboratory procedures in the case of semen quality. Existing studies on trends in semen quality have been retrospective and have reached different conclusions, and the issue remains controversial.

21. The IPCS Global Assessment [29] concluded that analysis of the human data, while generating concerns, has so far failed to provide firm evidence of direct causal associations between low level (general population) exposure to EDCs and adverse health outcomes. In part, this was due to limitations in the available data and the IPCS report highlighted a number of points, including:

- *"A number of studies report a decline in human sperm quality in several countries. The issue remains controversial. Even if there has been deterioration in semen quality, this would not necessarily be due to endocrine disruption.*"
- *"Available human and experimental animal studies demonstrate that high-level exposure to certain environmental chemicals can impair fertility and increase the risk of spontaneous abortion, but the relationship to endocrine disruption remains speculative*."
- *"Declining sex ratios (fewer males) have been recorded in a number of regions and countries and there is evidence that unidentified external influences are associated with such changes, but the mechanism(s) is unknown* ;"
- *"Temporal increases in the frequency of development abnormalities of the male reproductive tract, particularly cryptorchidism and hypospadias, have been reported, but the role of exposure to EDCs is unclear. Experimental data have shown that a number of chemicals can disrupt development of the male reproductive tract via endocrine mechanisms*."

Other possible risk factors

Possible causes of cryptorchidism

22. Causes of cryptorchidism are multiple and the precise etiological factors are still unknown; a recent review [55] has outlined a few of the risk factors. Some biomarkers, including a reported decrease in serum inhibin B levels and sperm concentration, have been associated with cryptorchidism [56].

23. Recently the role of insulin-like factor-3 (INSL3), which acts to retain the gonad in the inguinal region, has been highlighted in mice [57]. INSL3 expression in fetal testis is inhibited by maternal exposure to estrogens. Although to date no mutations have been found in the human INSL3 gene responsible for cryptorchidism, one associated mutation in the INSL3 receptor has been reported [58].

Lifestyle

24. An increasing number of publications are citing individuals' lifestyle aspossible risk factors for some of the adverse trends observed in the development of the male reproductive system [59, 60, 61]. These include advanced maternal age at birth of the first child, and low parity (number of siblings at the time of birth) [Error! Bookmark not defined., 59, 62].

25. An association between *in utero* exposure of the male fetus to maternal smoking, and reduction in semen quality [63] and testis size, has been reported [60, 61]. Diet and physical activity in adolescence have also been reported to be potential risk factors for the incidence of testicular cancer [64, 65]

Body size at birth and in adulthood

26. Positive associations have been reported for high birth weight [66], low birth weight (possibly due to intrauterine retardation) [59], height in young adulthood [67], and risk of testicular cancer, cryptorchidism and hypospadias. However, there is inconsistency between the studies.

Genetic factors

27. An increasing number of papers have focussed on the potential role of genetic factors and DNA damage, in the reported adverse trends in the development of the male reproductive system [68, 69]. Large DNA deletions on the Y chromosome are associated with infertility, such deletions remove genes crucial for the progress of normal spermatogenesis [70, 71]. It had been considered that the Y chromosome was particularly susceptible to genetic damage that will affect male fertility. However, there is now evidence, which suggests that the Y chromosome has developed a novel mechanism for dealing with its unique situation, in which mutations in single copy genes might arise. This involves a process termed gene conversion whereby multiple palindromic copies of genes exist within the Y chromosome, between which recombination may occur [72].

28. A recent study identified an association between male subfertility, and the chromosomal region 11p15 [69]. Mutations in the Zinc Finger genes, *ZNF214* and *ZNF215,* which are localised on chromosome 11p15, were found in patients (50% of whom reported a history of cryptorchidism) but not in the controls. These genes are predominantly expressed in the testis.

COT discussion

29. Although there is good evidence that the incidence of testicular cancer has increased, evidence for changes in sperm density, motility and morphology is less clear. Data on the quality of human sperm are subject to a number of sources of uncertainty, including analytical and methodological differences in assessment.

30. The Committee noted that extensive reviews had been conducted, including those of the IPCS [29], various other official bodies and academics. These have not provided convincing evidence that exposure to endocrine disrupting chemicals has adversely affected the human male reproductive system (in contrast to wildlife).

31. Efforts are being made to collect exposure data on various chemicals with endocrine disrupting potential. So far these have shown little correlation with reproductive effects. Furthermore the majority of these chemicals are of low potency compared to mammalian hormones. Although many of these compounds are persistent in the environment, exposure is generally very low. While some bioaccumulate in man, this is not true of all of them (e.g. phthalates).

32. The Committee agreed that there is a need for new approaches to consider possible causes of adverse trends in reproductive health, reported in some countries. In addition to endocrine disrupting chemicals, there is a need to consider whether chemicals without a direct effect on the endocrine system might be involved in the deterioration of male reproductive health, by some other toxic or genotoxic mechanism not mediated via classical hormone signalling mechanisms. Other possibly relevant factors include lifestyle changes that may have occurred in the last few decades, such as an increase in sedentary occupations and maternal age at first pregnancy, as well as decreased numbers of older siblings at birth (parity).

33. It was considered that although current animal studies demonstrate effects of EDCs on certain endpoints, such as maldescended testis, these data often cannot be extrapolated to humans and therefore may not be useful. For instance the poor quality of human sperm compared to that of animals may have implications when comparing human and animal data.

34. It was agreed that it would not be useful for the Committee to repeat the work of existing and ongoing international reviews and activity relating to endocrine disruption and adverse trends in the male reproduction system. There was a need for a new approach investigating the mechanisms involved in the formation of developmental abnormalities. The first step of the process would be to review the evidence for adverse trends in human male reproductive health, and then to consider possible causes including lifestyle factors and the role of chemicals in general. Since much of this would be outside of the Committee's terms of reference, this might best be explored by experts from appropriate medical and scientific disciplines at a scientific meeting organised by one or more learned societies.

Conclusions

35. We *note* that although the evidence of endocrine disruption in wildlife is more convincing, the extensive international reviews currently do not provide direct evidence that exposure to endocrine disrupting chemicals has adversely affected the human male reproductive system. This may be because of the

uncertainties involved in undertaking prospective long-term studies in humans.

36. We *consider* that the evidence of adverse trends in human male reproductive health should be reviewed first before considering possible causes. These could include the role of chemicals in general, not merely those affecting the endocrine system, as well as lifestyle factors.

37. Since much of this work is currently outside the Committee's remit, we *recommend* that a scientific meeting be held to review the evidence of adverse trends in male reproductive health. This will need to involve experts from relevant medical and scientific disciplines.

38. These conclusions may need to be reviewed as new data emerge.

COT statement 2004/04
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