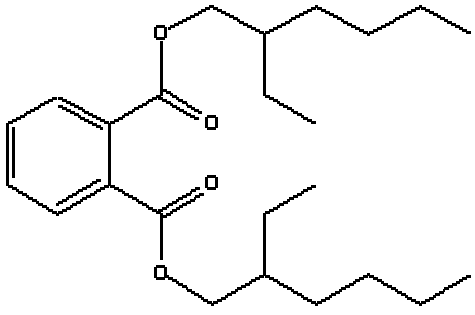
INTRA-UTERINE LIFE: A PERIOD OF MAXIMAL VULNERABILITY TO EXPOSURE OF MIXTURES OF MANMADE ENDOCRINE DISRUPTING CHEMICALS.

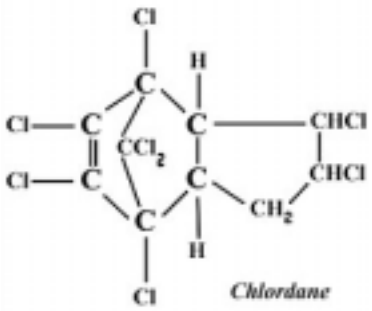
C. Vyvyan Howard, Developmental Toxico-Pathology Research Group, Department of Human Anatomy & Cell Biology, University of Liverpool

There is general agreement that high dose therapeutic exposure to synthetic hormones at critical stages of development can cause cancer, for example intrauterine exposure to diethylstilboestrol (DES) led to clear cell adenoma of the vagina in female offspring (Herbst *et al.,* 1971). However, a recurring argument against the causal link between environmental exposure to carcinogens and subsequent carcinogenesis is that pollutants are simply not present in sufficient environmental quantities to lead to a human exposure level that could cause cancer. Such conclusions, however, are usually based on cancer induction toxicology performed on adult laboratory animals. They also ignore the biological mechanisms that have evolved to minimise the bioavailability of naturally occurring hormones to the fetus. For example when a woman becomes pregnant, the blood levels of the protein that binds her natural oestrogen increase. Xeno-oestrogens do not usually bind efficiently to such binding proteins. Most of the chemicals of concern, which include phthalates, alkyl phenols, bisphenols, some pesticides, organo-metals and organochlorine POPs, have relatively low molecular weights and do not have difficulty crossing the placenta to reach the fetus.



DEHP: We know too little about its endocrine disrupting potency!

Lichtenstein et al (2000) have demonstrated that environmental rather than genetic factors are the major determinants in most cancers. Human evidence of links between exposure to environmental pollutants and cancer is beginning to emerge, as illustrated in a recent paper by Hardell *et al.* (Hardell *et al.,* 2003) which shows a positive correlation between higher current levels of PCBs, HCB and chlordanes (long lived pollutants) in mothers with sons who have developed testicular cancer.



Chlordane

A feature of human exposure to environmental chemical pollutants is the extreme complexity of the mixture of xeno-chemicals to which we are subjected, consisting of hundreds of chemical groups. If these groups are broken down into individual compounds, then there are tens of thousands of individual chemicals in the mixture. For example, the organo-chlorine pesticide toxaphene, which in many classifications would be regarded simply as one chemical, has over 62,000 theoretically possible variants if all congeners and enantiomers (mirror image isomers) are taken into account (Vetter, 1995; Vetter, 1998). Other groups of organic chemicals also have high numbers of variants. We simply do not possess the tools in toxicology to analyse complex mixtures (Howard, 1997). Even testing mixtures of two chemicals at a time is quite laborious (Axelrad *et al.,* 2002). There are some bio-monitoring methods being developed for estimating the total dioxin-like activity or oestrogen-like activity of mixtures (Murk, 1996; Soto *et al.,* 1997) but these do not identify individual components of the mixture. However, many of these components are acknowledged to be carcinogenic or cancer promoters.

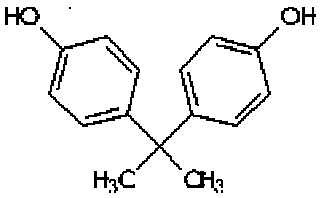
There are competing theories for the aetiology, or possible causal factors, of cancer. A frontrunner for nearly a century has been the somatic mutation hypothesis, attributed to Boveri, under which a chemical has to be a mutagen (either aneugenic or clastogenic) to be able to produce cancer (Boveri, 1929). This reliance on a single hypothetical genotoxic mechanism was questioned by

Peyton Rous in the 1950s and has been challenged by Sonnenschein and Soto in recent publications (Rous, 1959; Sonnenschein *et al*., 1999). Sonnenschein and Soto (1999) strongly argue in favour of revisiting the 'tissue organization field hypothesis' as a non-genotoxic cancer causation hypothesis. Under this hypothesis, it is proposed that all cells in the body are in a 'default' state of proliferation, as in other life forms, rather than 'quiescence', as previously assumed. In multicellular organisms, where cells must exist in an ordered 'society of cells', the desire to proliferate has become continuously suppressed by juxtacrine and autocrine (i.e. local hormonal) influences. This local control is intimately bound up with local architecture of the tissue at a microscopic level. The balance of the stroma to the parenchyma, and the relationship of one cell type to another are of considerable significance (Kaufman and Arnold, 1996; Maffini *et al*., 2004, Weaver and Gilbert, 2004). The tissue organization field theory proposes that carcinogens alter tissue interactions, such as those occurring between the stroma and the epithelium, that in turn cause the architectural changes found in carcinomas. The control of the micro-architecture of organs, throughout the developmental period, by epigenetic factors (external influences), is a delicate process. Some of these factors are known but most are not fully understood. Hormones, which are known to be involved in the control of organogenesis, act in concentrations in the low parts per trillion range. Xeno-chemicals, which may mimic hormones, are present in similar concentrations.

There are various examples of dysgenesis, or defective development, of organs or tissue, which constitute pre-malignant states, i.e. could cause cancer later. If undescended testes cannot be successfully repositioned surgically by orchidopexy, they are removed because there is a high risk of malignancy if they remain within the abdominal cavity, at a higher than optimal body temperature. The testis in these cases is dysplastic, i.e. has developed abnormally, though there is no suggestion of mutagenesis. Various types of testicular dysgenesis are on the increase within the background population, including cryptorchidism (Skakkebaek, 2001; Jacobsen, 2000; Moller, 1999). In addition, male patients who are subfertile have been shown to be more vulnerable to developing testicular cancer. Both conditions are related to testicular dysgenesis.

Vom Saal *et al.* (1992) have demonstrated the sensitivity of developing organs to physiological levels of natural hormones. They examined the effect of intrauterine position of the rat fetus on behaviour. They found that females developing between two sisters experienced ambient average oestradiol levels of 130 parts per trillion (ppt). However, females developing between two brothers experienced only 100 ppt of oestradiol. The latter were found to have a predictable life-long difference in behaviour. The female developed between two males was on average more aggressive, had fewer oestrous cycles and was 'less sexually appetising' than her counterpart who developed between two females. Vom Saal's hypothesis is that the minute difference in ambient oestradiol levels between the states, a mere 30 ppt, is sufficient to cause observed behavioural differences. If hormonal levels in the developing fetus are indeed that sensitive, it comes as little surprise to find that xeno-chemicals are able to modify the maturation of tissues during exquisitely sensitive periods of life.

The development of lactational ducts is modified in the mammary glands of laboratory rodents exposed in utero to environmentally relevant doses of the environmental oestrogen bisphenol-A (Markey *et al.,* 2001). This is an example, in an experimental setting, of low dose exposure to a known environmental pollutant causing tissue dysgenesis.



Bisphenol A

It is important to acknowledge that there will always be more than one explanation for a particular multifactorial human pathology. For example, a putative explanation attributed for the increase in human breast cancer incidence is that women are having fewer children and at a later age than would have been the case in pre-industrial times (Clavel-Chapelon, 2002), when much of a woman's fertile period would have been spent either pregnant or lactating.

There appears to be string evidence to support the hypothesis that, through the perturbation of organogenesis during critical stages of development, there is indeed a mechanism whereby seemingly low levels of exposure to hormonally-active substances could lead to the development of cancer later in life. This could occur at concentrations that would not necessarily affect adults. The implications of these observations will be discussed.

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Health-care devices. (Foto: **Moshammer**)