Institute of Science in Society
science society sustainability
ISIS

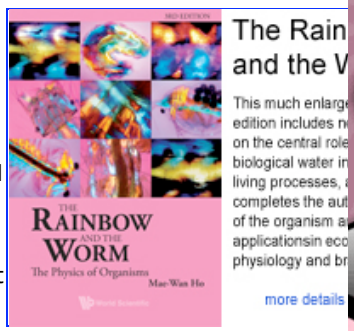
Epigenetics and Beyond

ISIS Press Release 21/01/09

Epigenetic Toxicology

Environmental toxins can modify our genes and affect our children and grandchildren; enormous implications for risk assessment of synthetic chemical and other xenobiotics Dr. Mae-Wan Ho

Bisphenol A in baby bottles and other plastics cause developmental defects across generations



A common chemical bisphenol A (BPA) that leaches out from baby bottles and other containers of food and drink, when given to pregnant mice, caused gross chromosomal defects in the egg cells of her female foetuses. These female foetuses grew into adults and produced large proportions of defective embryos [1]. It was a case of the grandmother effect spanning three generations.

BPA is a weak oestrogen mimic, and one of the most abundant synthetic chemicals manufactured by the chemical industry; some 2 to 3 million tonnes made every year .It is the monomer base of polycarbonate plastic food and drinks containers, including baby bottles, the resin lining of tins and dental sealants; it is also found in 'carbonless' paper used for receipts as well as a wide range of other common household products. BPA has been linked to a range of reproductive defects in lab rodents, including reduced sperm production, and increased susceptibility to prostate cancer in the male, and abnormalities in mammary gland, brain development and the oestrous cycle in the female. Foetal exposure to BPA in rats was shown to induce the development of precancerous and cancerous lesions in the mammary gland [2]. In humans, an association between BPA levels and recurrent miscarriages has been suggested [3], and recently, a significant relationship was found between urine concentrations of BPA and cardiovascular disease, type 2 diabetes, obesity, and liver enzyme abnormalities in a representative sample of the adult US population [4].

The US research group led by Patricia Hunt at Washington State University, Pullman, made headlines around the world when they published the results in 2007 [1]. They also found that knocking out the oestrogen receptor ERb in transgenic mice reproduced precisely those defects caused by BPA, suggesting that BPA interfered with the binding of oestrogen to its receptor ERb. And indeed, no further abnormalities could be induced by BPA in the ERb knock-out mice.

These results provide evidence for a multigenerational effect of BPA exposure mediated by the dysregulation of ERb, so the daughters of exposed pregnant females have an increased risk of producing offspring with abnormal chromosomes. The BPA exposure also adversely influences the reproductive lifespan of the females, as these abnormalities typically result in the loss of a significant proportion of oocytes in developing female foetuses prior to sexual maturation.

The findings of Hunt's team [1] that really attracted the attention of the scientific community were the epigenetic effects (resulting from interference of oestrogen binding to its receptor) transmitted across generations, which have become

Recent Publications

NEW! The Rainbow and the Worm, The Physics of Organisms
"Probably the Most Important Book for the Coming Scientific Revolution" Now in its Third Edition
Buy Now|More info

NEW! Food Futures Now:
*Organic
*Sustainable
*Fossil Fuel Free
How organic agriculture and localised food (and energy) systems can potentially compensate for *all* greenhouse gas emissions due to human activities and free us from

increasingly important in toxicology.

During foetal development, the germ cells in both sexes undergo massive proliferation by ordinary cell division. Thereafter, germ cells in the testis of the male foetus stop dividing and remain in developmental arrest until after birth, while those in the ovary of the female foetus start meiosis (the special cell division that leads to the formation of egg cells with half the chromosome number). By the time of birth, the oocytes (as they are now called) have entered a protracted period of meiotic arrest where they remain until just prior to ovulation after an extensive period of growth in the adult ovary.

Since the publication of these findings, the Canadian federal government has formally declared BPA a hazardous substance, and announced they would seek to restrict imports, sales and advertising of polycarbonate baby bottles containing BPA [5].

In contrast, a battle is still raging in the US, where the Federal Drugs Administration (FDA) is widely criticised for ignoring its own panel of scientific advisors and siding with the American Chemistry Council (industry's lobby group) in insisting that BPA is not harmful at current exposure levels.

In Europe, similarly, the European Food Safety Authority has repeatedly reaffirmed its opinion that BPA is 'safe', dismissing all scientific evidence on grounds of 'reliability' and 'consistency'.[6].

The toxic effects of BPA should be considered along with other hormone disruptors such as DES and a host of pesticides in the context of the new discipline of epigenetic toxicology. Human epidemiologic evidence reveals that individuals exposed to DES *in utero* during the first 3 months of pregnancy showed increased incidences of reproductive disorders and the rare cancer, clear cell adenocarcinoma of the vagina. Increased incidences of uncommon disorders were also found in the granddaughter and grandsons of DES-exposed women, suggesting epigenetic inheritance [7]. This has been confirmed in experiments on rats (see later).

The evidence that adult health and disease is influenced by the environment of foetal or early neonatal development is not unique to endocrine disruption. Low birth size and poor nutrition have been associated with increased risk of heart disease, type 2 diabetes, osteoporosis and metabolic dysfunction [8]. Gestation represents a developmental window of vulnerability to epigenetic changes by nutritional and environmental factors [9] (see Epigenetic Inheritance, "What Genes Remember", *SiS* 41). There is a mounting body of evidence from both gene-specific and genome-wide studies that environmental exposures particularly in early development can induce epigenetic changes that may be transmitted to subsequent generations and/or lead to diseases in later life.

Toxicogenomics' referred to a combination of conventional toxicology, the study of poisons especially their effects of organisms and the ecosystem, with genomics, the study of the function of nucleotide sequences in the genomes of organisms [10]. It has contributed immensely to defining the adverse biological effects of environmental stressors, toxins, drugs and chemicals; but is rapidly being transformed into 'epigenetic toxicology'; here defined as potentially heritable changes in gene expression *with*, or without accompanying alterations in the DNA sequence of the genome [9].

Epigenetic mechanisms are necessary for normal development and differentiation, but these can be misdirected, leading to diseases, notably cancers.

The epigenetic progenitor model of cancer and mechanisms

Inherited and spontaneous or environmentally induced epigenetic alterations are increasingly recognized as early molecular events in cancer development, as summarised in a review published in *Cancer Journal* in 2007 [11], which also described a new epigenetic model of cancer, involving stem cells. *Stem cells*, present in all multi-cellular organisms, are capable of renewing themselves through cell division and are also pluripotent, i.e., capable of giving rise to a diverse range of differentiated specialized cells [12] (see Hushing Up Adult Stem Cells, *SiS* 13/14).

Cancer arises in three steps [11]. First, an epigenetic alteration of stem/progenitor cells within a tissue, affecting genes that regulate the expansion of progenitor cells and increase their capacity for self-renewal and pluripotency. For example, loss of regulation of *IGF2* promotes an expansion of the progenitor cell compartment, increasing the probability of tumour formation. This alteration can be due to events within the stem cells themselves, the influence of the stromal compartment (the supporting framework of connective tissues in and around organs), or environmental damage or injury.

Second, a gatekeeper mutation occurs in a tumour-suppressor gene (in solid tumours), or rearrangement of an oncogene. At the third and final stage, genetic and epigenetic instability set in, leading to increased tumour evolution.

Epigenetic alterations are potentially more damaging than nucleotide mutations because their effects on regional chromatin structure can spread, thereby affecting multiple genetic loci (units, usually of transcription). Furthermore, they tend to affect a high proportion of those exposed, unlike conventional gene mutations, which are relatively rare. The most commonly described changes in cancer are alterations in the methylation pattern of DNA, but epigenetic modifications of histone proteins are also implicated.

DNA methylation, the covalent addition of a methyl group to the C5 position of cytosine, is involved in many key developmental processes, deregulation of which would result in major developmental abnormalities. These include the inactivation of alleles in imprinted genes, genes that are expressed or not at different times according to whether they come from the mother or the father; inactivation of one of the two X chromosomes in the female to compensate for the male that has only one X chromosome; and in suppressing transposable elements, which would otherwise jump around and disrupt genes and genome function in general.

DNA methylation occurs most frequently in CpG islands, and results in a conformational change in the major groove of the DNA that alters protein binding. CpG islands are regions in the genome with a high GC content and frequent CpG occurrence. The human and mouse genome projects identified ~15 500 and ~29 000 CpG islands respectively. Hypermethylation of CpG-rich regions of gene promoters inhibits expression by blocking the initiation of transcription.

Transcription of a number of tumour suppressor genes such as *p16* ^{INK4a}, *BRCA1*, *p53*, and *hMLH-1* were found to be inhibited by promoter hypermethylation in cancers.

Although silencing of tumour suppressor gene by DNA methylation occurs frequently in cancer, genome-wide *hypo*methylation is one of the earliest events in the genesis of cancer. Demethylation of the genome can lead to the reactivation of transposable elements, thereby altering the transcription of adjacent genes, the activation of oncogenes such as *H-RAS*, and inappropriate expression of imprinted genes. Furthermore, genomic instability associated with the hypermethylation of the DNA mismatch repair enzyme gene *MLH1* may deregulate not only critical genes involved in the initial stages of carcinogenesis, but also those involved in the later invasion and metastasis stages.

Two distinct sets of genes that potentially link environmental exposures during pregnancy to adult disease susceptibility are imprinted genes and genes with metastable expression states. Imprinted genes are inactivated according to the parent of origin, so that only one of the two alleles (forms of a gene) should be functional. Metastable *epi*alleles (epigenetic forms of an allele) have highly variable expression because of stochastic changes in their epigenetic state, in at least some cases such as the mouse *Agouti* gene, due to the insertion of a virus like intracisternal A particle.

Imprinted genes can be deregulated in both germ cells and somatic cells. Because imprinted genes are frequently clustered and their expression co-ordinately regulated by imprinted control regions, a single genetic or epigenetic change in an imprinted control region can result in disrupting many genes. Consequently, imprinted genes are associated not only with severe developmental disorders such as Angelman, Beckwith-Widemann, and Prader-Willi syndromes, but also with cancer. Imprinted genes are at much greater risk of inactivation by mutation and epigenetic alteration because one allele is already inactivated by genomic imprinting. Because imprinted genes normally encode either positive or negative growth effectors, they are frequently involved in the formation of a wide range of tumours.

The epigenome is particularly susceptible to disturbance of regulation by environmental factors during gestation, neonatal development, puberty, and old age. Age-correlated increases in DNA promoter methylation occur in a number of genes involved in cancer, including *IGF2*. *Versican*, and *PAX6*. Alterations in epigenotype have also been observed after adult exposure to xenobiotic chemicals (synthetic chemicals not normally produced or metabolised by organisms).

Studies in animals provide clear evidence of epigenetic inheritance of disease states for generations after the initial exposure. I describe two cases involving hormone disrupting chemicals.

Fungicide caused high incidence of illness up to four generation after exposure

The anti-androgenic compound vinclozolin is a fungicide mainly used on oilseed rape and peas in the UK and on vines, fruit and vegetables worldwide [13]. It was first introduced by BASF in Germany in 1976 and sold under a number of trade names including Ronilan and Flotilla. Vinclozolin is linked to testicular tumours in rats, and reproductive toxicity, and the UK Advisory Committee on Pesticides has kept it under review since 1991, while US Environment Protection Agency considers it an endocrine-disrupting chemical. Vinclozolin is metabolized into more

active compounds with higher binding affinity to the androgen receptor.

Researchers at Washington State University Pullman in the United States led by Michael Skinner found that exposure of rats during embryonic development at the time of sex-determination resulted in adult animals from the F1, and subsequent generations up to F4, developing a number of diseases or abnormalities of the prostate, kidney, immune system, testis, and tumours in various tissues. In addition, several blood abnormalities developed including high cholesterol levels.

High incidences of trans-generational disease states were found, consistently across all generations, and appeared to be due in part to epigenetic alterations in the male germ-line, and successive studies carried out confirm the effects [14].

High doses were administered at 100mg/kg/day from embryonic day 8-14 of gestation, with no further treatment in subsequent generations, and inbreeding was avoided. So only the F1 embryos and F2 germ cells (in the F1 embryos) were exposed.

The diseases and abnormalities were scored 'blind', i.e., the scorer did not know if the rats were in the group that had been exposed to the chemical.

Testis abnormalities included atrophied tubules, vacuoles or failure of germ cell formation at a rate of 20 percent or greater. Kidney lesions include tubular damage at 30 percent or more, tubular changes involved extreme dilation with protein-rich fluids, fluid-filled cystic tubules, thickening of the Bowman's capsule surrounding the glomerulus, as well as reduced glomerular area. Ventral prostate tissues were considered abnormal if more than 30 percent of prostate ducts atrophied and contained no columnar secretary epithelial cells. Immune related abnormalities include excessive macrophage and lymphocyte invasion into multiple organs and generally accompanied by bacterial infection. Several types of inflammation were identified in the inner ear, lower limbs, and lower respiratory tract. Sub-dermal abscesses were found which grew in size and caused septicaemia (widespread infection).

There were no tumours in control animals while tumours developed in 12-33 percent of the animals in the F1-F4 generation exposed animals: 4 breast adenomas, 2 breast carcinoma, 1 lung sarcoma and 2 skin Merkel cell melanoma together with the breast adenoma; only the lung sarcoma, Merkel cell tumour and breast carcinoma were malignant. Prostate lesions were detected in 45-55 percent of males. Renal lesions appeared in 20 to 50 percent of males of all vinclozolin-exposed generations, and were also found in females. Abnormal testis function and morphology were found in 15-38 percent of the F1-F4 generations of exposed animals. The pathology included increase in spermatogenic cell death, gross morphological defects in spermatogenesis, and a complete lack of spermatogenesis.

Inflammation of the inner ear, sub-dermal abscesses and bacterial infection were found in 12-33 percent of exposed animals, with no inflammation in controls. Significant increase in cholesterol was found in 35 percent of exposed animals at 6-14 months old, but not at 3 months.

Up to 50 percent of exposed animals also developed an apparent premature ageing with appearance and behaviour at 6-14 months similar to controls older than 18 months.

The frequency of disease in the F1 generation was often less than subsequent generations. Many animals had multiple abnormalities, and 85 percent of all F1-F4 vinclozolin-exposed animals developed a transgenerational disease state. Over 90 percent of all males in F1-F4 vincozolin-exposed group had a reduced capacity of making sperms.

The disease phenotype was primarily transmitted through the male germ-line; a vinclozolin treated F2 male out-crossed to a wild-type female gave an increase in disease prevalence over controls in the F3 generation (though less than the vinclozolin F3), whereas the reverse cross did not. But the female germline also contributed to disease.

Although tumours, renal lesions and prostate lesions are observed in aged (24 mo) rats, none of these were observed in the controls at 6-14 mo, when the two groups of animals were compared.

The results were highly significant, and the frequencies of defects were much higher than could be accounted for by mutations rates, which are typically 1 to 5 percent. The researchers had used an exposure level that is almost 10 times the official lowest level at which adverse effects were observed, i.e., 11 mg/kg/day; but biological effects have been demonstrated at doses around 1mg/kg/day. Environmental levels of vinclozolin have not yet been rigorously determined.

Analysis of the sperm from the F2 and F3 generations identified 25 candidate DNA sequences with altered methylation patterns in the vinclozolin-exposed animals, with 15 sequences confirmed to have specific hypermethylation [15]. These

sequences mapped to specific genes and non-coding DNA regions. The expression pattern of several of the genes during embryonic development was altered in the vinclozolin F1 and F2 generation testis, with some decreased in expression while others were increased.

All candidate genes altered in the vinclozolin samples were not in the controls. They are present on various autosomes with no major hot spot regions, and none was present on the sex chromosomes.

Synthetic oestrogen causes cancer in descendants of exposed female animals

The synthetic oestrogen diethylstilbestrol (DES) is a potent prenatal endocrine disruptor. Exposure in humans and experimental animals during critical periods of reproductive tract development in embryogenesis permanently alters oestrogen target tissues and results in long-term abnormalities such as cancer of the uterus later in life.

For almost 30 years, DES was prescribed to women with high-risk pregnancies to prevent miscarriages and other complications. In 1971, a clinical report associated DES with a rare form of reproductive tract cancer, vaginal adenocarcinoma, which was detected in a small number (0.1 percent) of adolescent daughters of women who had taken DES while pregnant [16]. Subsequently, DES was also linked to more frequent benign reproductive tract problems in an estimated 95 percent of the DES-exposed daughters; reproductive organ dysfunction, abnormal pregnancies, reduced fertility and disorders of the immune system. Similarly, male offspring of exposed females showed structural, function, and cellular abnormalities in the reproductive organs, inflammation, and decreased fertility [17].

DES is no longer used clinically to prevent miscarriage, but the exposed are still to reach an age where higher incidence of cancers are expected, and the possibility of second-generation effects has been reported, i.e., in the grandchildren of women prescribed DES, as mentioned earlier.

Recent research in the United States led by Retha Newbold at the National Institutes of Environmental Health Sciences of the National Institute of Health, North Carolina, found that in mice, exposure to DES leads to altered gene expression that includes an oestrogen-regulated component [18]. DES is a model endocrine disruptor and even low doses increase the incidence of cancer in the uterus, as do low doses of other environmental oestrogen. The increased propensity to develop tumours is transmitted through the mother to subsequent generations of male and female descendants. The mechanisms include both genetic and epigenetic events.

Neonatal mice (day 1-5) treated with DES at 2mg/pup/d developed a high incidence of cancer (90-95 percent) at 18-24 months of age. These tumours rarely spread throughout the body, but in aged animals (24 mo or older) the lesions spread to para-aortic lymph nodes or extended to contiguous organs. Similar findings were obtained in rats and hamsters. This may be predictive of carcinogenic potential of environmental estrogens in women as they age, as both the histology of the tumours and the progression to cancer are similar to those seen in women.

In a dose response study, DES was found to cause tumours even at a dose of 0.0002mg/pup/day. Uterine cancer followed a linear dose-dependent response from 0 in controls to 65 percent at 2mg) as did increase in uterine weight/body weight (estrogenicity) (01 in control to 0.3 at 2 mg/pup/day)

Other environmental oestrogens also caused uterine lesions: 17b-estradiol, tamoxifen, hexestrol, tetrafluorodiethylstilbestrol, ethinyl estradiol, 2-hydroxyestradiol, 4-hydroxyestradiol, genistein, nonylphenol, bisphenol A, methoxychlor. Most were tested at 2mg/pup/day, except the weaker estrogens, genistein, nonylphenol, bisphenol A and methoxychlor, which were administered at 200 mg/pup/d. All except methoxychlor caused uterine lesions in aged mice. Methoxychlor failed to cause lesions possibly because neonate liver cannot yet convert it into oestrogen.

Prenatal DES exposure was found to delay the expression of *Hox* genes involved in the development of the reproductive tract. *Wnt* genes were also affected. Neo-natal exposure caused demethylation of the oestrogen-responsive gene *LF* in the mouse uterus, which may be involved in tumour induction.

In hamsters exposed to DES, similarly, uterine carcinoma develop at a high frequency, and imbalances in the oestrogen-regulated uterine expression of *c-jun*, *c-fox*, *c-myc*, *bax*, *bcl-2* and *bcl-x* proto-oncogenes probably played a role.

Microarray studies with mouse uterus revealed similar altered gene expression pathways that included an oestrogen-regulated component.

In a further series of experiments, prenatal or neonatal treatment with DES led to tumours in the female and male genital tract, and in addition, the susceptibility for tumours was transmitted to the descendants through the maternal germ cell lineage. Transmission via the DES-exposed male was not studied.

The mice were treated with DES prenatally at 2.5, 5 or 10 mg/kg/dy on day 9-16 of gestation, or at 0.002 mg on day 1-5 after birth; these were the highest doses that did not drastically interfere with fertility later in life. When F1 female mice reached sexual maturity, they were bred to control untreated males. Female and male offspring were aged to 17-24 months and examined for genital tract abnormalities. An increased incidence of proliferative lesions of the rete testis (a network of ducts in the centre of the testis associated with the production of sperms and an oestrogen target tissue in the male), and tumours of the reproductive tract were observed in the male offspring. In female offspring, an increased incidence of uterine adenocarcinoma was seen. The incidence was lower in DES descendants than in their parent, (31 percent in F1 at 18 months at neonatal dose of 0.002 compared with 11 percent in their F2 descendants). Several genes were permanently dysregulated after DES treatment. The oestrogen-responsive proteins LF and c-fos were permanently up-regulated in the uterus, and the promoter regions of these genes were hypomethylated. LF was also over-expressed in uterine tissues from female offpring of the DES exposed females.

It is important, therefore, to follow the grandchildren of women exposed to DES.

Large implications for public health and risk assessment of xenobiotics

These findings in epigenetic toxicology have large implications for public health and the risk assessment of xenobiotics. It is estimated that more than 100 000 xenobiotics are on the market in the European Union, some 70 000 are potentially hazardous for human health and/or the ecosystem [19]. The vast majority of these chemicals have not been adequately tested for safety before they were released. Many of them are endocrine disruptors similar to those whose epigenetic effects have been reviewed in this article. Common household products – detergents, disinfectants, plastics and pesticides – contain endocrine disruptors. For example, 56 pesticides in use have been identified as known or suspected endocrine disruptors by the European Union and the scientific community [20], but are still largely unregulated.

The new findings call for urgent action on two fronts. First, all known and suspected endocrine disruptors and carcinogens should be banned or phased out where there is overwhelming scientific evidence of harm, or the precautionary principle, where there is reasonable suspicion of harm. Pesticides and chemical fertilizers are good candidates for phasing out, if not a total ban, as there is now substantial evidence that organic agriculture, which dispenses with pesticides and other chemical inputs, works on all scales, and compost and green manure can maintain or indeed, *increase* yields over chemical fertilizers [21] (see Food Futures Now *Organic *Sustainable *Fossil Fuel Free, ISIS publication).

Second, an adequate protocol involving transgenerational studies with microarray analyses for genetic and epigenetic effects should be used in all toxicological investigations.

One class of xenobiotics that must be included are genetically modified food and feed, for which epigenetic effects have already been demonstrated in exposed animals, as well as health impacts in both laboratory feeding studies and on livestock and farm workers in farmers' fields [22-24} (*GM is Dangerous and Futile*. *SiS* 40; GM Maize Reduces Fertility & Deregulates Genes in Mice, and GM Maize Disturbs Immune System of Young and Old Mice, *SiS* 41).

References

1. Susiarjo M, Hassold TJ, Freeman E and Hunt PA. Bisphenol A exposure in utero dispurts early oogenesis in the mouse. PLoS Genetics 3(1), e5. doi:10.1371/journal.
2. Murray TJ, Maffini MV, Ucci AA, Sonnenschein C and Soto AM. Induction of.mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. *Reprod Toxicol* 2007, 13, 383-90.
3. Sugiurra-Ogasawara M, Ozaki Y, Sonta S, Makino T. and Suzumori K. Exposure to bisphenol A is associated with recurrent miscarriage. *Hum Reprod* 20, 2325-9.
4. Lang IA, Galloway TS, Scarlett A et al. Association of urinary bisphernol A concentration with medical disorders and laboratory abnormalities in adults. *JAMA* 2008, 300(11), 1303-10.
5. Bisphenol A. Wikipedia.26 December 2008, http://en.wikipedia.org/wiki/Bisphenol_A
6. Bisphenol A (BPA) – its use within the food and veverage industry and safety concerns Questions & answers. The Facts. Australian Bottled Water

Industry, accessed 1 January 2009, http://www.bottledwater.org.au/scripts/cgiip.exe/WService=ASP0003/ccms.r?PageId=10051
7. Newbold R. Lessons learned from perinatal exposure to diethylstilbestrol. Toxicol Appl Pharmacol 2004, 32, 213-9.
8. Barker DJ. Components in the interpretation of the high mortality in the county of Finnmark. *Int J Epidemiol* 2002, 31, 309-10.
9. Ho MW. Epigenetic inheritance, "what genes remember". Science in Society 41 (to appear).
10. Reamon-Buettner SM, Mutschler V and Borlak J. The next innovation cycle in toxicogenomics: environmental epigenetics. *Mutation Res* 2008, 639, 158-65.
11. Weidman JR, Dolinoy DC, Murphy SK and Jirtle RL. Cancer susceptibility: epigenetic manifestation of environmental exposures. *Cancer J* 2007, 13, 9-16.
12. Ho MW and Cummins J. Hushing up adult stem cells. i-sis news13/14, 6-7, 2002. http://www.i-sis.org.uk/HUASC.php
13. Vinclozolin, Pesticide News 50, December 2000, http://www.pan-uk.org/pestnews/Actives/Vinclozo.htm
14. Anway MD, Leathers C and Skinner MK. Endocrine disruptor vinclozolin induced epigenetic transgenerational adult onset disease. *Endocrinology* 2006, 14 September,
15. Chang HS, Anway MD, Rekow SS and Skinner MK. Transgenerational epigenetic imprinting of the male germ-line by endocrine disruptor exposure during gonadal sex determination. Endocrinology. 14 September 2006 doi:10.1210/en.2006-9087
16. Herbst AL, Ulfelder H and Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. N Engl J Med. 1971, 284, 878-81.
17. Giusti RM, Iwamoto K, and Hatch II. Diethylstilbestrol revisited: a review of the long-term health effects. *Ann Intern Med* 1995, 122, 778-88.
18. Newbold RR, Padilla-Banks E and Jefferson WN. Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinology* 2006, 147, S11-S17.
19. COST European Cooperation in the Field of Scientific and Technical Research, Memorandum of Understanding for the implementation of a concerted European Research Action designated as COST Action 636 "Xenobiotics in the Urban Water Cycle", 17 February 2005, http://register.consilium.eu.int/pdf/en/05/st00/st00218.en05.pdf
20. Harriott N and Feldman J. Pesticides that disrupt endocrine system still unregulated by EPA. Pesticides and You 2008, 28, 11-14.
21. Ho MW, Burcher S, Lim LC, et al. *Food Futures Now, Organic, Sustainable, Fossil Fuel Free*, ISIS and TWN, London, 2008
22. Ho MW. GM *is* dangerous and futile. Science in Society 40, 4-8, 2008.
23. Ho MW. GM maize reduces fertility and deregulates genes. Science in Society 41 (to appear).
24. Ho MW. GM maize disturbs immune system of young and old mice. Science in Society 41 (to appear).

The Institute of Science in Society, The Old House 39-41 North Road, London N7 9DP
telephone: **[44 20 7700 5948] [44 20 8452 2729]**

Contact the Institute of Science in Society