

Agricultural chemicals and endocrine-mediated chronic toxicity or carcinogenicity

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This brief review summarizes information on the endocrine effects and mechanisms of action of certain pesticides and considers whether exposure to pesticides with endocrine activity may play a role in human endocrine-related tumors of the breast, testis, prostate, and endometrium. Both animal and human data are considered. If animal data are to be used effectively for predicting human risk, a thorough understanding of comparative endocrinology and the underlying endocrine and pathological mechanisms contained in the animal model is needed. It is concluded that the evidence does not support an association between organochlorine pesticides and breast cancer, while the evidence on other tumor sites is too sparse to draw any conclusions concerning pesticides.

Key terms animal; atrazine; breast; endometrium; cancer; human; organochlorine; pesticide; prostate; review. testis.

The last few years have seen an explosion of research interest in chemicals, both natural and manmade, that can act as endocrine disruptors, together with much speculation about whether exposure to such chemicals via the environment and the food chain may have adverse effects on wildlife and humans (1–10). Attention has focused on the potential effects of endocrine disruptors on wildlife populations, and, in laboratory animal and human studies, on reproduction, neurobehavior, immune function, and the development of cancer. These systems are considered to be susceptible to endocrine disruption because their development or later functioning in adult life is known to be influenced by exposure to endogenous chemicals or xenobiotics with endocrine activity. Endocrine-related mechanisms are involved in the development of certain human cancers and the possible role of agricultural chemicals in endocrine-mediated carcinogenicity needs to be considered.

Laboratory studies on pesticides with endocrine activity

Several pesticides have been shown to interact with endocrine receptors in vitro or to have endocrine-mediated effects in laboratory animals in vivo, as shown in table 1. The potential for disrupting sex hormones has

been the most studied. There are examples of pesticides that have estrogenic activity, and, while there are no known examples of pesticides with androgenic activity, there are several which possess anti-androgenic activity. Some have both estrogenic and anti-androgenic activity. Some act peripherally at the cellular enzyme or hormone receptor level, and others act centrally via the brain. The European Union has listed 40 pesticides among several hundred candidate substances suspected of interfering with the hormone systems of humans and wildlife (15).

Observation of activity in vitro does not necessarily imply that the compounds will be active in vivo. For example, dieldrin and endosulfan, which are both estrogenic in vitro, are negative in the uterotrophic assay in immature rats and do not affect pituitary weight or pituitary levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone, or thyroid stimulating hormone in immature rats (16–18).

In contrast to many other putative endocrine disrupting chemicals, many pesticides have been tested in rodent multigeneration studies, which are capable of detecting endocrine disruptors that are active in vivo on sex hormones or their receptors, particularly if conducted according to more recently updated protocols [Organisation for Economic Co-operation and Development (OECD). Detailed review paper: appraisal of test methods for sex-hormone disrupting chemicals. OECD;

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Table 1. Biological evidence that pesticides can act via endocrine mechanisms. [LH = luteinizing hormone, *o,p'*-DDT = 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane, *p,p'*-DDE = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene]

Effects	Endocrine mechanism	Examples
Estrogenic in vitro (receptor binding or activation, breast cancer cell proliferation) or estrogenic in vivo (female reproductive tissues, uterotrophic assay)	Estrogen receptor agonists (ER α , β)	Chlordane, <i>o,p'</i> -DDT, dieldrin, endosulfan, fenarimol, toxaphene, methoxychlor (metabolite) (10)
Inhibition of male mating behavior; inhibition of parturition	Inhibition of aromatase (converts testosterone to estradiol centrally and peripherally)	Fenarimol, prochloraz, endosulfan (11)
Delayed puberty; disturbed estrous cycles; reduction in embryo implants; earlier onset of mammary tumors	Acts on the hypothalamus to suppress LH and prolactin surges	Atrazine (10, 26, 12)
Anti-androgenic effects (reduced anogenital distance, retained nipples, hypospadias, reduced testis and accessory sex organ weight, reduced sperm count and fertility, delayed puberty)	Androgen receptor antagonists	<i>p,p'</i> -DDE, fenarimol, fenitrothion, linuron, methoxychlor (metabolite), prochloraz, procymidone, vinclozolin (10, 11, 13, 14)
Anti-androgenic effects (reduced prostate growth)	Inhibition of 5 α -reductase (converts testosterone to dihydrotestosterone)	Atrazine (22)

2002. OECD series on Testing and Assessment no 21. [http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono\(2002\)8](http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/env-jm-mono(2002)8); US Environmental Protection Agency (US EPA). Endocrine disruptor screening and testing advisory committee (EDSTAC) final report. US EPA; 1998. <http://www.epa.gov/scipoly/oscpendo/edsreview/finalrpt.htm>. The general similarities in physiological and pathological processes between laboratory mammalian species and man are such that the default assumption is that animal data should be considered relevant for human risk assessment. Nevertheless, extrapolation from in vivo studies on animals may sometimes be inappropriate for human risk assessment. In the field of endocrine disruption, knowledge of comparative endocrinology and the mechanism of action of an endocrine-active chemical are prerequisites for deciding whether animal data are relevant for human risk assessment. This situation can be illustrated with the herbicide atrazine.

Observations indicate that atrazine is an endocrine disruptor in rat, with some strain differences in responses. Atrazine does not bind to rat or human estrogen receptors (19), is inactive in the human breast cancer cell line (MCF-7 cells) proliferation assay (20), and is inactive in the uterotrophic assay in adult ovariectomized Sprague-Dawley (SD) rats (19). However, exposure of weanling Wistar rats delayed puberty in both genders (21, 22). Estrous cycles were disturbed in adult SD, Long Evans (LE), and Fischer 344 (F344) rats, with an increased time in diestrus, albeit with higher and longer duration doses required for SD rats (23). Atrazine has been shown to act on the hypothalamic-pituitary axis to suppress LH and prolactin surges in LE rats but not in SD rats (24), presumably via a reduction in gonadotropin-releasing hormone (GnRH).

SD rats have a high background incidence of mammary tumors. In 2-year bioassays in which atrazine was given at 400 ppm in the diet, an increased incidence of

mammary tumors with earlier onset than normal was observed in SD rats but not in F344 rats (25, 26). The proposed mechanism for the strain difference is that reproductive aging occurs earlier in the SD rat than in the F344 rat (25). In SD rats there would be a reduced release of GnRH with aging, leading to reduced FSH and LH release, which in turn leads to delayed ovulation, persistent vaginal cornification, and consequent increased exposure to endogenous estrogens and an increase in the ratio of estradiol to progesterone (10). However, it is very unlikely that such a mechanism would be relevant to women since, at menopause, FSH levels increase and estrogen levels decrease (27).

Human observations

There are only a few known examples of adverse effects on humans after high exposures to endocrine-disrupting chemicals, and, in the case of cancer, these effects are related to exposure to human pharmaceuticals, such as estrogens and androgens, rather than to low-level exposures to environmental chemicals. Nevertheless, the possible effects of low-level exposures raise important public health questions because even small shifts in population distributions of adverse health outcomes could potentially have considerable impact on the overall health of large populations.

In the media, much emphasis in the debate on endocrine disruptors has been placed, for example, on the increasing global trends for breast cancer and testicular cancer incidence. However, lack of adequate data on human exposures to potentially relevant chemicals severely hampers the exploration of hypotheses on the possible causes of any identified trends. In particular, the lack of exposure data on critical periods of development that influence later functioning in life make it

difficult to determine causative associations between exposure and effect.

Breast cancer

The background for the suspicion that environmental endocrine disruptors could play a role in breast cancer has been described in detail elsewhere (10). Briefly, the incidence of breast cancer has been rising steadily since the 1940s. There are marked geographic differences in incidence, high in western Europe and North America and low in Asia. There is a clear influence of environment and lifestyle, deduced from observations that the breast cancer risks of migrants become similar to that of the host country. There is a clear role for estrogens in the etiology of breast cancer. The risk of breast cancer is related to lifetime exposure to endogenous estrogens (estimated 30–50% of total) and to exposure to exogenous estrogens, such as those in oral contraceptives and hormone replacement therapy. Some genotypes are at high risk for breast cancer (eg, BRCA1, BRCA2), and it has been speculated that persons with such genotypes may be more susceptible to any effects from estrogenic environmental chemicals.

Many investigations have been made into the relationship between exposure to organochlorine pesticides and breast cancer. There have been 34 publications since 1976 (of which 32 were published in 1990–2001) on nonoccupational exposure to dichloro-diphenyl-trichloroethane (DDT) or dichlordiphenylethylene (DDE) and breast cancer, which have been cited and described in a recent review by the International Programme on Chemical Safety (IPCS) (10). In summary, four small case-control studies have found a significant association between breast cancer and adipose tissue concentrations of *p,p'*-DDE, and one larger case-control study showed a significant trend based on serum concentrations of *p,p'*-DDE. However, nine prospective case-control studies have failed to show any relationship with serum concentrations of *p,p'*-DDE, and twelve retrospective case-control studies have failed to show a relationship with serum or adipose tissue concentrations of *p,p'*-DDE. Two retrospective case-control studies did show a positive relationship with serum concentrations of *p,p'*-DDE. A preliminary study on occupational exposure to high levels of DDT found no link to breast cancer (28).

Dieldrin has also been investigated. Two case-control studies showed no relationship between breast cancer and serum or adipose tissue concentrations of dieldrin (29, 30). One study showed a nonsignificant increase in the high versus low tertile for plasma dieldrin concentrations, but a test for trend was significant (31).

The relationship between breast cancer and hexachlorobenzene (HCB) exposure has also been studied.

Ten case-control studies, cited and described in the IPCS review (10), showed no significant association with serum or breast adipose tissue concentrations of HCB. One case-control study showed a nonsignificant, twofold increase in risk when the high versus low tertile serum HCB participants were compared; a test for trend was significant for women diagnosed within <3 years of blood collection, but not for those diagnosed >3 years after blood collection (30).

In rodent carcinogenicity bioassays, DDT or DDE, dieldrin, and HCB are positive for liver (and some other) tumors in mice, rats, and hamsters, but no increases in mammary tumors have been observed (32). Overall, the human and experimental evidence does not support an association between exposure to organochlorine pesticides and an increased incidence of breast cancer.

Testicular cancer

The incidence of testicular cancer has been increasing by 2–4% a year since the 1960s among men under 50 years of age in developed countries (33). The start of the increase can be traced back to the 1920s (34). There are marked differences in incidence, even between countries located close to each other; for example, the incidence in Denmark is fourfold higher than that in Finland. There are also marked differences between races; Caucasians have a threefold higher incidence than African Americans.

A biologically plausible link between prenatal exposure to estrogens and the development of testicular cancer has been hypothesized (35). However, there are few published epidemiologic studies on testicular cancer and xenoestrogen exposure. The sons of mothers exposed to the powerful estrogen diethylstilbestrol (DES) during pregnancy have a twofold increased risk of developing testicular cancer, and this increase was significant in a meta-analysis (33). There was no association between human adipose *p,p'*-DDE concentrations in white males in the United States and testicular cancer 2–22 years later (36). Rodent models of testicular cancer are inappropriate for human risk assessment since rodents have Leydig cell tumors, whereas human seminomas develop from abnormal germ cells (37). To date, there are insufficient data on exposure to endocrine disruptors in general, or pesticides in particular, and the occurrence of testicular cancer with which to draw any conclusions.

Prostate cancer

Other endocrine-related tumors, such as prostate cancer and endometrial cancer, have been even less well studied with respect to any relationship with exposure to endocrine disruptors. Prostate cancer is the

most commonly diagnosed cancer among men in developed countries (38). Age-adjusted incidence rates have increased abruptly since the 1980s probably due to improved screening and diagnosis. There are marked differences in incidence between races, African Americans having a higher incidence than Caucasians and Asians having the lowest incidence (39). The tumors are clearly androgen-dependent and modulated by estrogen therapy. Epidemiologic studies have shown no significant association between prostate cancer and exposure to polychlorinated biphenyls, dioxins [2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)], or DDE (10). For Canadian farmers, a weak but significant association between acres sprayed with herbicides and deaths from prostate cancer has been reported (40). The relevance of animal models to human prostate cancer is unclear.

Endometrial cancer

Risk factors for endometrial cancer are similar to those for breast cancer, unopposed estrogen being the major risk factor. However, in contrast to breast cancer, there is no increasing temporal trend for endometrial cancer. Epidemiologic studies on environmental and occupational exposure to organochlorine compounds, including DDT, are negative (10). Experimental studies have shown that neonatal exposure to DES causes uterine cancer in mice (41). The pesticides toxaphene and methoxychlor have been shown to cause uterine hyperplasia in rats (42, 43), and atrazine and vinclozolin cause uterine adenocarcinoma in rats [44 & unpublished report by Mellert W. Report: carcinogenicity study with reg. no. 83248 (vinclozolin) in rats: administration in the diet for 24 months. Unpublished report from BASF AG, Ludwigshafen, Germany, 1995. Submitted to WHO by BASF AG, Ludwigshafen, Germany. Cited in Pesticide residues in food—1995, part II—toxicological and environmental, IPCS, p 379–404.] The very limited human data on exposure to endocrine disruptors and the occurrence of prostate cancer or endometrial cancer do not allow any conclusions to be drawn.

Concluding remarks

Given that endocrine-mediated mechanisms are known causes of some cancers in humans, the possibility that exposure to pesticides with endocrine activity may contribute to cancer incidence has to be considered. Both environmentally persistent pesticides, several of which are no longer in use, and some pesticides still in current use have been demonstrated to have endocrine-mediated toxicities in laboratory studies. However, to date, there is no convincing evidence that exposure to pesticides at

environmentally encountered concentrations (including diet) is playing a causal role in human cancers. It should be noted that, for the purposes of exploring relationships between endocrine disruptors and tumors, there are major deficiencies in the data available to date. There have been few measurements of exposure to potentially relevant chemicals in epidemiologic studies on human cancer, and there are even fewer studies addressing exposure at potentially relevant time periods early in life. Some animal models are also of questionable relevance.

References

1. Colborn T, Clement C. Chemically-induced alterations in sexual and functional development; the wildlife/human connection. Princeton (NJ): Princeton Scientific Publishing Co; 1992.
2. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, et al. Male reproductive health and environmental chemicals with estrogenic effects. Copenhagen: Danish Environment Protection Agency; 1995.
3. MRC Institute for Environment and Health. IEH assessment on environmental oestrogens: consequences to human health and wildlife. Leicester (UK): MRC Institute for Environment and Health; 1995.
4. German Federal Environment Agency. Endocrine active chemicals in the environment (texte 3/96). Berlin: Umweltbundesamt; 1996.
5. European Commission. European workshop on the impact of endocrine disruptors on human health and wildlife, Weybridge, UK. Brussels: European Commission DG XXI; 1996. Environment and Climate Research Programme, report no EUR 17549.
6. Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaatari S, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA sponsored workshop. Environ Health Perspect 1996;104 Suppl 4:715–40.
7. Ankley GT, Johnson RD, Toth G, Folmar LC, Detenbeck NE, Bradbury SP. Development of a research strategy for assessing the ecological risk of endocrine disruptors. Rev Toxicol B Environ Toxicol 1997;1:231–67.
8. US Environmental Protection Agency (US EPA). Special report on environmental endocrine disruption: an effects assessment and analysis. Washington (DC): US EPA; 1997. Report no EPA/630/R-96/012, February (Risk Assessment Forum).
9. National Research Council (NRC). Hormonally active agents in the environment. Washington (DC): NRC, National Academy Press; 1999.
10. Damstra T, Barlow S, Bergman A, Kavlock R, Van der Kraak G, editors. Global assessment of the state-of-the-science of endocrine disruptors. Geneva: International Programme on Chemical Safety (IPCS), World Health Organization; 2002. WHO/PCS/EDC/02.2.
11. Andersen HR, Vinggaard AM, Rasmussen TH, Gjermansen IM, Bonefeld-Jorgensen EC. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity *in vitro*. Toxicol Appl Pharmacol 2002;179:1–12.
12. Cummings AM, Rhodes BE, Cooper RL. Effect of atrazine on implantation and early pregnancy in 4 strains of rats. Toxicol

- Sci 2000;58:135–43.
13. Tamura H, Maness SC, Reischmann K, Dorman DC, Gray LE, Gaido KW. Androgen receptor antagonism by the organophosphate insecticide fenitrothion. *Toxicol Sci* 2001;60:56–62.
 14. McIntyre BS, Barlow NJ, Wallace DG, Maness SC, Gaido KW, Foster PM. Effects of *in utero* exposure to linuron on androgen-dependent reproductive development in the male Crl:CD(SD)BR rat. *Toxicol Appl Pharmacol* 2000;176:87–9.
 15. Commission of the European Communities (CEC). Communication from the Commission to the Council and the Parliament on the implementation of the Community Strategy for Endocrine Disruptors (COM (1999) 706)—a range of substances suspected of interfering with the hormone systems of humans and wildlife. Brussels: CEC; 2001.
 16. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL. Assessing environmental chemicals for estrogenicity using a combination of *in vitro* and *in vivo* assays. *Environ Health Perspect* 1996;104:1296–300.
 17. Wade MG, Desaulniers D, Leingartner K, Foster WG. Interactions between endosulfan and dieldrin on estrogen-mediated processes *in vitro* and *in vivo*. *Reprod Toxicol* 1997;11:791–8.
 18. Ashby J, Lefevre PA, Odum J, Harris CA, Routledge EJ, Sumpter JP. Synergy between synthetic estrogens? *Nature* 1997;385:494.
 19. Tennant MK, Hill DS, Eldridge JC, Wetzel LT, Breckenridge CB, Stevens JT. Chloro-s-triazine antagonism of estrogen action: limited interaction with estrogen receptor binding. *J Toxicol Environ Health* 1994;43:197–211.
 20. Conner K, Howell J, Chen I, Liu H, Berhane K, Sciarretta C, et al. Failure of chloro-s-triazine derived compounds to induce estrogen receptor-mediated responses *in vivo* and *in vitro*. *Fundam Appl Toxicol* 1996;30:93–101.
 21. Laws SC, Ferrell JM, Stoker TE, Schmid J, Cooper RL. The effects of atrazine on female Wistar rats: an evaluation of the protocol for assessing pubertal development and thyroid function. *Toxicol Sci* 2000;58:366–76.
 22. Stoker TE, Laws CE, Guidici DL, Cooper RL. The effect of atrazine on puberty in male Wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol Sci* 2000;58:50–9.
 23. Cooper RL, Stoker TE, Goldman JM, Parrish MB, Tyrey L. Effect of atrazine on ovarian function in the rat. *Reprod Toxicol* 1996;10:257–64.
 24. Cooper RL, Stoker TE, Tyrey L, Goldman JM, McElroy. Atrazine disrupts the hypothalamic control of pituitary-ovarian function. *Toxicol Sci* 2000;58:297–307.
 25. Stevens JT, Breckenridge CB, Wetzel LT, Gillis JH, LuemPERT LG III, Eldridge JC. Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides. *J Toxicol Environ Health* 1994;43:139–53.
 26. Wetzel LT, LuemPERT III LG, Breckenridge CB, Tisdell MO, Stevens JT, Thakur AK, et al. Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley rats and Fischer 344 rats. *J Toxicol Environ Health* 1994;43:169–82.
 27. Vom Saal FS, Finch CE, Nelson JF. Natural history and mechanisms of reproductive aging in humans, laboratory rodents, and other selected vertebrates. In: *The physiology of reproduction*. 2nd edition. New York (NY): Raven Press; 1994. p 1213–314.
 28. Cocco P, Blair A, Congia P, Saba G, Ecça AR, Palmas C. Long-term health effects of the occupational exposure to DDT: a preliminary report. *Ann NY Acad Sci* 1997;837:246–56.
 29. Wassermann M, Nogueira DP, Tomatis L. Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. *Bull Environ Contam Toxicol* 1976;15:478–84.
 30. Dorgan JF, Brock JW, Rothman N, Needham LL, Miller R, Stephenson HE Jr, et al. Serum organochlorine pesticides and PCBs and breast cancer risk: results from a prospective analysis (USA). *Cancer Causes Control* 1999;10:1–11.
 31. Hoyer AP, Jorgensen T, Brock JW, Hartvig HB. Repeated measurements of organochlorine exposure and breast cancer risk (Denmark). *Cancer Causes Control* 2000;11:177–84.
 32. Wolff MS, Collman GW, Barrett JC, Huff J. Breast cancer and environmental risk factors: epidemiological and experimental findings. *Ann Rev Pharmacol Toxicol* 1996;36:573–96.
 33. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillelte LJ Jr, et al. Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 1996;104 Suppl 4:741–76.
 34. Bergstrom R, Adami HO, Mohnher M, Zatonski W, Storm H, Ekblom A, et al. Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. *J Natl Cancer Inst* 1996;88:727–33.
 35. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 1993;341:1392–5.
 36. Cocco P, Benichou J. Mortality from cancer of the male reproductive tract and environmental exposure to the anti-androgen p,p'-dichlorodiphenyldichloroethylene in the United States. *Oncology* 1998;55:334–9.
 37. Cook JC, Klinefelter GR, Hardisty JF, Sharpe RM, Foster PM. Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms and relevance to humans. *Crit Rev Toxicol* 1999;29:169–261.
 38. Parker SL, Tong T, Bolden S, Wingo RJ. Cancer statistics, 1997. *CANADIAN Cancer J Clin* 1997;47:5–27.
 39. Crisp TM, Clegg ED, Cooper RL, Wood WP, Anderson DG, Baetcke KP, et al. Environmental endocrine disruption: an effects assessment and analysis. *Environ Health Perspect* 1998;106 Suppl 1:11–56.
 40. Morrison H, Savitz D, Semenciw R, Hulka B, Mao YL, Morrison D, Wigle D. Farming and prostate cancer mortality. *Am J Epidemiol* 1993;137:270–80.
 41. Newbold RR, Banks EP, Bullock B, Jefferson WN. Uterine adenocarcinoma in mice treated neonatally with genistein. *Cancer Res* 2001;61:4325–8.
 42. Reuber MD. Carcinogenicity of lindane. *Environ Res* 1979;19:460–81.
 43. Reuber MD. Carcinogenicity and toxicity of methoxychlor. *Environ Health Perspect* 1980;36:205–19.
 44. International Agency for Research on Cancer (IARC). Occupational exposures in insecticide application and some pesticides. Lyon: IARC; 1991. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol 53. p 179–250.