On the rumors about the silent spring. Review of the scientific evidence linking occupational and environmental pesticide exposure to endocrine disruption health effects

Rumores de uma primavera silenciosa: uma revisão das evidências científicas sobre a associação entre exposição ocupacional e ambiental a pesticidas e distúrbios endócrinos

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Abstract *Occupational exposure to some pesticides, and particularly DBCP and chlordecone, may adversely affect male fertility. However, apart from the therapeutic use of diethylstilbestrol, the threat to human reproduction posed by "endocrine disrupting" environmental contaminants has not been supported by epidemiological evidence thus far. As it concerns other endocrine effects described in experimental animals, only thyroid inhibition following occupational exposure to amitrole and mancozeb has been confirmed in humans. Cancer of the breast, endometrium, ovary, prostate, testis, and thyroid are hormone-dependent, which fostered research on the potential risk associated with occupational and environmental exposure to the so-called endocrine-disrupting pesticides. The most recent studies have ruled out the hypothesis of DDT derivatives as responsible for excess risks of cancer of the reproductive organs. Still, we cannot exclude a role for high level exposure to o,p'-DDE, particularly in post-menopausal ER+ breast cancer. On the other hand, other organochlorine pesticides and triazine herbicides require further investigation for a possible etiologic role in some hormone-dependent cancers.*

Key words *Reproduction; Antithyroid Agents; Neoplasms; Pesticides; Endocrine Disruptors*

Resumo *A exposição ocupacional a determinados pesticidas, particularmente ao DBCP e à clordecona, pode ter efeitos adversos sobre a fertilidade masculina. Entretanto, com exceção do uso terapêutico do dietil-estilbestrol, a ameaça à reprodução humana através da "desregulação endócrina" por contaminantes ambientais ainda não foi comprovada através de evidências epidemiológicas. A questão diz respeito a outros efeitos endócrinos descritos em animais experimentais, e apenas a inibição tireóide foi confirmada em seres humanos, após exposição ocupacional a amitrole e mancozeb. O fato de serem hormônio-dependentes os cânceres de mama, endométrio, ovário, próstata, testículos e tireóide motivou pesquisas sobre o risco potencial associado à exposição ocupacional e ambiental aos pesticidas conhecidos como "desreguladores endócrinos". Os estudos mais recentes descartaram a hipótese dos derivados do DDT como responsáveis pelo risco em excesso de câncer dos órgãos reprodutivos. Entretanto, não se pode excluir o papel da exposição elevada ao o,p'-DDE, particularmente no câncer de mama pós-menopáusica, positivo para receptores estrogênicos. Além disso, há necessidade de mais investigação sobre o possível papel etiológico de outros pesticidas organoclorados e herbicidas triazínicos em alguns cânceres hormônio-dependentes.*

Palavras-chave *Reprodução; Antitiroidianos; Neoplasias; Praguicidas; Desreguladores Endócrinos*

Endocrine system and pesticides

Experimental studies

Reproductive effects of pesticides have received special attention among the investigators. The discovery that organochlorines, such as hexachlorocyclohexane (HCH) γ- and δ-isomers, dichlorodiphenyl-trichloroethane (DDT) isomers *p,p'*-DDT and *o,p'*-DDT, its *p,p'*-DDE derivative, methoxychlor (or dianisyl-trichloroethane, a structural analogue of DDT), dieldrin, and pentachlorophenol, as well as atrazine, a widely used nitrogen herbicide, bind *in vitro* to the rat androgen receptor, significantly inhibiting the specific binding of [3H]5 α-dihydroxytestosterone (DHT) (Kelce et al., 1995), further fostered research on the issue. Such an anti-androgen effect was mostly prominent for *p,p'*-DDE (Fent, 1997; Kelce et al., 1995). Besides, the *o,p'*-DDT levo enantiomer (Chen et al., 1997; Dees et al., 1997; Fent, 1997; McBlain & Lewin, 1976), nonylphenol, and to a lesser extent also methoxychlor and pentachlorophenol, but not β-HCH (Steinmetz et al., 1996), link to the rat estrogen receptor significantly reducing *in vitro* [3H]17 β-estradiol binding (Danzo, 1997). Also, alachlor, trans-nonachlor, endosulfan, and atrazine competed with [3H] 17 β-estradiol for binding to the alligator estrogen receptor (ER), while endosulfan, alachlor, and kepone (also known as chlordecone) inhibited the binding of the synthetic progestin [3H]R5020 to alligator pregesterone receptor (aPR) (Vonier et al., 1996). The γ isomer of HCH, on the other hand, did not have an effect on the binding of radiolabelled estradiol to the uterine endometrial explants in bovine, as derived from the effects on DNA synthesis. The uterine response to *o,p'*-DDT administration in immature rats is similar to that elicited by 17 β-estradiol, with an increase in DNA synthesis and cell division in the luminal epithelium, stroma and myometrium (Robison et al., 1985). However, the maximum response following *o,p'*-DDT treatment varies by cell type, from 70% of that produced by 17 β-estradiol in stroma and myometrium, to the same though delayed maximum response in the luminal epithelium (Robison et al., 1985). Consistently, *o,p'*-DDT and methoxychlor produce the uterine hyperplasia characteristic of estrogens, although with the magnitude and timing of the response is dependent on the specific cell type observed, the concentration, and the animal species (Tiemann et al., 1996; Ulrich et al., 2000), and mice treated with *o,p'*-DDT and β-HCH, at blood concentrations equal to or above 18ng/ml and 42ng/ml respectively, showed increased uterine epithelial height and vaginal epithelial thickness compared to control animals (Ulrich et al., 2000). Also, *o,p'*-DDT inhibits the binding of 3H-estradiol to the 8-9S estrogen binding protein of rat testicular cytosol, while *p,p'*-DDE does not, and methoxychlor requires metabolic activation (Bulger et al., 1978; Cummings, 1997). In fact, its didemethylated metabolite 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTE) causes marked suppression of 3H-estradiol binding (Bulger et al., 1978; Cummings, 1997). When administered orally to pregnant mice from days 11-17 of pregnancy, *o,p'*-DDT and methoxychlor reduced significantly the rate of urine marking by male offspring in adulthood at the highest dose administered prenatally (vom Saal et al., 1995). Relative binding to estrogen receptors in MCF-7 cells accurately predicted the doses of *o,p'*-DDT and methoxychlor that produced the same results, providing support for the hypothesis that effects on behavior were mediated by binding to estrogen receptors in the developing brain (vom Saal et al., 1995). It has been reported that *o,p'*-DDE could transactivate the human Estrogen Receptor (hER) in MCF-7 and T-47D human breast cancer cells with a 140- to 300-fold weaker potency than that of estradiol (Kupfer & Bulger, 1977; McBlain, 1987), eliciting an additive response when given together with estradiol (McBlain, 1987). These concentrations were considered close or around the range of concentrations among exposed human populations. *O,p'*-DDT induces an estrogen-inducible protein indistinguishable from that formed after 17 β-estradiol, without additional induction over that seen with maximum levels of the natural estrogen, further supporting the premise that these compounds share a common pathway in stimulating the synthesis of induced protein (Robison et al., 1984). DDT isomers and metabolites may also stimulate other estrogenic endpoints in estrogen-responsive MCF-7 cells, such as the induction of the progesterone receptor, the inhibition of the progesterone-induced reporter gene activity in a dose-dependent manner through both hPR-dependent and hPR-independent pathways, and the down-regulation of the hER (Chen et al., 1997; Klotz et al., 1997b; Mason et al., 1980).

Estrogenic properties, as derived from the proliferative effect in the MCF7-E3 human breast cancer cell model, have been described also for Toxaphene (or polychlorocamphene) (Soto et al., 1994; Stelzer & Chan, 1999), a mixture of over 800 congeners, largely used as an

insecticide in the United States until 1982, primarily to control insect pests on cotton and other crops, on livestock and to kill unwanted fish in lakes. Other organochlorine pesticides, such as dieldrin, and endosulfan revealed estrogenic properties comparable to those of DDT and chlordecone in the MCF7-E3 model. In this assay, when mixed together, estrogenic chemicals may act cumulatively inducing estrogenic responses at concentrations lower than those required when each compound is administered alone (Soto et al., 1994). Other pesticides, such as the carbamate insecticides aldicarb, Baygon (propoxur), bendiocarb, carbaryl, methomyl, and oxamyl demonstrated a limited capacity to displace radiolabeled estrogen or progesterone from ER or PR in whole cell competition binding assays (Klotz et al., 1997a). Parathion, possibly the most widely used organo-phosphorous insecticide, interfered with normal differentiation of A/Snell mice testes, implanted in the allantochorion of chicken eggs treated with the pesticide, causing a complete disorganisation of the seminiferous cords and the testicular interstitium (Rojas et al., 1998).

Pesticides may interfere with sexual hormones also through indirect non receptorial mechanisms (Fent, 1997). For instance, *o,p'*-DDT stimulates rat uterine contraction in a fashion not dependent on Prostaglandin E2 release or direct estrogen receptor-related action (Juberg & Loch-Caruso, 1992) and chlordecone is capable of inhibiting sexual behavior in rodents, but this effect does not depend on an attenuation of estradiol-dependent elevation of CNS progesterone receptors (Eckols et al., 1989). Nonylphenol, and to a lesser extent also HCH, *o.p'*-DDT and pentachlorophenol, reduce [3H]5 α-DHT binding to the human sex hormone-binding globulin (hSHBG) (Danzo, 1997). Organo-tin compounds inhibit cytochrome P-450 dependent monoxygenases, such as aromatase, which oxidizes testosterone to estradiol (Fent, 1997). In these two last instances, the resulting effect is an increase of the quote of serum free testosterone available to link the androgen receptor, i.e. a pro-androgen effect. Activation of liver microsomial enzymes by organochlorines such as *o,p'*-DDT, *p,p'*-DDE, Chlordecone and Mirex, causes a dose and time dependent increase in estrogen metabolism through 2-hydroxylation of estradiol (Britton, 1975; Bulger & Kupfer, 1983). Treatment with DDT, and to a lesser extent methoxychlor, markedly induced the cytochromes P4502B1/2B2 and 3A activity, but not CYP2E1 or the P450 reductase activity, in treated rats (Li et al., 1995). Therefore, the resulting increase in testosterone and methoxychlor hydroxylation was related to induction of certain P450 enzymes, and not to enhanced reductase activity. It has been subsequently shown that CYPs induction by DDT differs by gender, with a 18-fold increase in CYP3A2 activity among female Wistar rats versus a non significant less than 3-fold induction in males (Sierra-Santoyo et al., 2000). As CYP3A2 is androgen dependent, the authors inferred that DDT is capable of modulating sexual metabolic dimorphism. CYP2B1/2B2 activity also showed a 19-fold increase in both genders. However, as no correlation exists between the ability to induce hepatic microsomal estradiol-2-hydroxylase activity and estrogenic (or antiestrogenic) properties of a given compound (Britton, 1975; Bulger & Kupfer, 1983), it is not clear whether and to what extent such *in vivo* indirect effects could add to or counterbalance the direct receptorial effects of hormone-like pesticides. Also, due to the multiple *in vivo* targets of the same pesticide often resulting in opposite end points, differences between *in vitro* and *in vivo* assays are to be expected. Further difficulties in extrapolating from experimental results to forecast human health effects arise from the dose of the toxicant administered to the experimental animal, which is several orders of magnitude greater than that resulting from occupational or environmental exposure, and from humans experiencing multiple exposures at the same time (such as with diet or drinking water) or close in time (such as in agricultural occupations). To match the peculiarities of human exposure to pesticides, real life mixtures of pesticides at concentrations similar to those found in contaminated groundwater in Iowa (alachlor, atrazine, cyanazine, metolachlor, metribuzin, and ammonium nitrate) and California (aldicarb, atrazine, dibromochloropropane, 1-2 dichloropropane, ethylene dibromide, simazine, and ammonium nitrate) were administered to Swiss CD-1 mice (Chapin & Gulati, 1997; Heindel et al., 1997). No detectable reproductive effects were observed, although animals treated with the California pesticide mix showed on average a 11% reduction in seminal vesicle weight (Chapin & Gulati, 1997).

Epidemiological studies

Comparison of occupations among couples seeking artificial insemination with donor sperm because of poor sperm quality versus couples treated by in-vitro fertilization due to female causes revealed a significantly greater

prevalence of agricultural occupations among spouses with male factor infertility, who also reported more long-term exposure to numerous insecticides and other pesticides (Strohmer et al., 1993). Findings among Danish pesticide sprayers did not confirm a generalized risk in farming occupations, as their median sperm concentration was not statistically different from unexposed men, and there were not significant changes in the sperm morphology, vitality, motility, sperm chromatin denaturation, and reproductive hormones following pesticide exposure (Larsen et al., 1999). Other studies have evaluated fecundability, i.e. the ability to obtain conception within a menstrual cycle (Olsen, 1994), or the "time to pregnancy" (TTP) index, i.e. the time with unprotected intercourse before achieving pregnancy (Baird et al., 1986; Joffe, 1997), as the outcome in relation to pesticide exposure. Results were also contradictory. Spouses of Dutch fruit growers, mostly exposed to the fungicide captan, showed a significant reduction of the fecundability ratio, particularly when the couple had tried to start pregnancy in the period when pesticides were applied, and in coincidence with numerous indicators of high level pesticide exposure (De Cock et al., 1994). Spouses of Italian greenhouse workers had a significantly longer TTP (5.4 months, *sd* 5.6) compared to spouses of white collar workers (3.9 months, *sd* 3.1), and TTP was mostly prolonged among workers who did not use personal protective equipment and among those heaviest exposed (Petrelli et al., 2000b). On the other hand, no effect of pesticide exposure on male fertility, evaluated with the adjusted fecundability ratio in the spouse, was observed among Danish and French greenhouse workers, vineyard workers, and other agricultural workers (Thonneau et al., 1999a, 1999b). Also, a Canadian study did not find a consistent pattern of association of pesticide exposure with time to pregnancy (Curtis et al., 1999). Only during exposure intervals in which women participated in pesticide activities (although in most instances the men also participated), a decrease in fecundability was observed related to the herbicides dicamba, glyphosate, and 2,4-D, and to organophosphates, thiocarbamates, and carbaryl. The conditional fecundability ratio ranged 0.51-0.80, but none was statistically significant (Curtis et al., 1999). It is unclear whether an endocrine mechanism or an increase in the spontaneous abortion rate in the earliest period of pregnancy due to other factors, such as teratogenic effects of some pesticides, might account for the decrease in fecundability observed in some studies. However, although not relevant from a Public Health perspective, such a possibility is worth to be considered for the medical diagnosis of infertility, defined as a waiting time of 12 months or more before conception, which showed a 3-fold increase in risk among women exposed to pesticides (Smith et al., 1997). Also, male-mediated risk of spontaneous abortion showed a 3.8-fold increase and the ratio of spontaneous abortions/pregnancies was 0.27 among the wives of 51 Italian pesticide applicators, whose exposure list included fenthion, DDVP, chlordane, DDT, dieldrin, lindane, malathion, and trichlorfon, compared to the wives of 51 food retailers, as the referents (spontaneous abortions/pregnancies ratio = 0.07) (Petrelli et al., 2000a).

The epidemiological inquiry into the reproductive effects of individual chemicals in agricultural settings is particularly difficult, and results sometimes poorly interpretable, due to the extremely complex pattern of exposure, resulting from the yearly use of a large number of individual chemicals for the same crop, the frequent variety of crops raised in the same farm, and the change in type of crops and chemical treatments along the years. Nonetheless, dibromochloropropane (DBCP), mostly used as an insecticide in banana plantations, was identified as a significant reproductive hazard causing infertility and sterility in men (Whorton et al., 1979). Azoospermia and oligospermia was largely above expectation among workers in DBCP manufacturing plants, with evidence of a relationship between duration of employment and effect on testicular function. Also, a lower fertility level and sterility were reported among agricultural workers exposed to DBCP in Costa Rica banana plantations (Potashnik & Porath, 1995). Plasma follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were significantly increased and testosterone level was not significantly decreased among the DBCP severely affected individuals (Kharrazi et al., 1980). Since removal of DBCP exposure, recovery of fertility was reported among some men who showed improvements in sperm counts, while others remained either azoospermic or severely oligospermic (Whorton, 1994). Such an inter-individual variation in the response might be related to polymorphisms of metabolizing genes, such as cytochrome P4502E1, the glutathione S-transferases m and q, and the paraoxonase genes (Au et al., 1999). Also, an Israeli study of DBCP exposed banana workers showed a threefold increase in spontaneous abortion (Kharrazi et al., 1980), while there was no increase in the

rate of spontaneous abortions and congenital malformations among pregnancies conceived during or after DBCP exposure in a more recent study (Potashnik & Porath, 1995). However, a low prevalence of male infants conceived during paternal exposure was found as compared with the pre-exposure period (16.6% versus 52.9%; $p < 0.025$). Restoration of fertility was followed by a gradual increase of this value to 41.4% (Potashnik & Porath, 1995). No adverse effect on sperm count was reported for DBCP exposure within the current OSHA standard (1 ppb) (Whorton, 1994).

Other individual pesticides have been less frequently evaluated in human studies. Kepone (chlordecone) has been shown to affect semen quality among severely poisoned individuals (Whorton, 1994), and changes in sperm characteristics were reported among Indian agricultural workers with long term exposure to ethylene dibromide (Ratcliffe et al., 1987). However, some problems in the study design and poor definition of exposure prevent meaningful interpretation of the latter report (Whorton, 1994). Serum LH concentrations were significantly increased among 54 male workers compared to 20 administrative clerks in a lindane producing factory (Tomczak et al., 1981). Blood levels of testosterone and FSH were not affected by lindane exposure in this study. Following reproductive toxicology studies in rats of molinate (a thiocarbamate herbicide used for weed control in rice fields), sperm count and reproductive history of 272 molinate formulation and production workers at three United States plants was studied, and exposure monitored (Tomenson et al., 1999). Sperm count and serum hormone levels were not related to molinate exposure nor was there evidence of reduced fertility among these workers. Also, no evidence of hormonal responses, and particularly anti-androgenic effects, was reported among 67 men exposed to vinclozolin, a widely used organic nitrogen fungicide, during synthesis and formulation operations compared to 52 controls (Zober et al., 1995). This study was set in response to concern raised by the results of animal studies, showing increased testes weight and decreased prostate, seminal vesicle and epididymis weights following oral administration of vinclozolin. More recent reports focus on an anti-androgenic effect of this fungicide during male differentiation (Monosson et al., 1999; Wolf et al., 2000), which would not be observed in an occupational setting. A Danish study of male greenhouse workers explored semen quality and sexual hormones in relation to exposure to a list of 60 pesticides (Abell et al., 2000). The most frequently reported chemicals included the insecticides pirimicarb, methomyl, deltamethrin, and endosulfan, and the fungicides benomyl, iprodione, and chlorothalonil. Only exposure to the general category of pesticides was evaluated in relation to sperm concentration, motility, morphology and viability and to plasma levels of testosterone, SHBG, FSH, and LH. None of these outcomes was affected by intensity of pesticide use. However, estimates of current dermal exposure and years of greenhouse work were inversely related to sperm concentration, motility, and viability, and to testosterone plasma levels, while the opposite was observed with the mean LH plasma level and current dermal exposure, and with the mean FSH plasma level and years of greenhouse work.

While occupational exposure to some pesticides, and particularly DBCP, may result in a definite reproductive hazard, which did not raise special public concern, a disproportionately heated debate continues in the scientific and non scientific press about the threat to human reproduction posed by environmental exposure to "endocrine disruptors". A review of 61 international studies on sperm count and semen volume combined the reported findings in a linear regression against time, and reached the conclusion that a significant decrease had occurred from 1938-1990 (Carlsen et al., 1992). A French study published thereafter reported a 2.1-2.6% annual decrease in sperm concentration, along with a decrease in mobility and normal spermatozoa rates among healthy men volunteering for sperm donation in more recent years (Auger et al., 1995). Similar findings were reported in Scotland (Irvine, 1994), and Belgium (Van Waeleghem et al., 1994), but not in Finland (Suominen & Vierula, 1993). It has been questioned whether these studies indicate that substantial changes in human fertility are occurring overall (Olsen, 1994), and it is questionable whether a decrease in sperm count, when still above levels of about 40 million/ml, may affect fecundability (Bonde et al., 1999). However, the connection with early and more recent observations in wildlife from contaminated areas opened the way to speculation about a role of organochlorines in causing a worldwide decline in human fertility (Sharpe, 1995; Skakkebaek et al., 1998), although temporal and spatial variability of human fertility do not seem associated with environmental exposure to DDT derivatives (Cocco, 1997; Safe, 2000). Also, it has been postulated that exposure to environmental "estrogenic" chemicals during pregnancy may induce the develop-

ment of reproductive abnormalities (e. g. cryptorchidism and hypospadia) and a reduction in sperm count of the male offspring (Seibert, 1997). Findings among United States populations of the Great Lakes area, consuming sport fish contaminated with PCBs and chlorinated pesticides, such as DDE, hexachlorobenzene, and mirex, were contradictory (Buck et al., 1997, 2000). A significant decrease in the fecundability ratio was reported for maternal consumption of PCBs contaminated fish lasting 3-6 years in one study (Buck et al., 2000). To date, apart from the therapeutic use of diethylstilbestrol, no link with other environmental contaminants has been supported by epidemiological evidence (Pottern et al., 1997). However, this does not exclude an endocrine-etiology for some reproductive adverse effects resulting from human exposures to specific pesticides.

If occupational or environmental exposure to xenoestrogens were a plausible human reproductive toxicant through receptorial mechanisms, dose and estrogenic potential with reference to the natural estrogen would be crucial, as all experimental animal studies have confirmed. The E-screen assay has been proposed to test the estrogenic effect of chemicals on estrogen sensitive human breast cancer cell lines, such as MCF-7 cells. This test is based on the dose-related estrogen-dependent proliferation of MCF-7 cells during 6 days of culture, with reference to estradiol (Soto et al., 1995; Toppari et al., 1995). The E-screen assays produces two outcomes: (1)the relative proliferative potency (RPP), which is the ratio between the lowest estradiol concentration required to yield maximal proliferation and the lowest concentration of the test compound needed to achieve the same effect; and (2) the relative proliferative effect (RPE), which is 100 times the ratio between the maximal cell yield obtained with the test compound and that obtained with estradiol. As shown in Table 1, estradiol can induce maximal cell yields at concentrations ranging 10-100 picomoles, while xenoestrogen pesticides achieve comparable effects at doses two orders of magnitude higher than estradiol. Estradiol is produced by the ovary in the amount of 100-200mg/day, and it can be released by dermal patches used as hormone replacement therapy in the menopause in the amount of 50mg/day. The normal range of estradiol blood concentration varies in the pre-menopausal women depending on the phase of the menstrual cycle, from 30-120pg/ml in the follicular phase, to 90-330pg/ml in the ovulatory period, and to 65-180pg/ml in the luteal period. Values in the post-menopausal women range 10-50pg/ml, and values in adult men range 15-70pg/ml (Yen, 1991). Based on these results of the E-screen assay, in order to compete significantly with estradiol ER binding, *o,p'*-DDE blood concentration would have to be 30-180µg/ml in fertile women and 10-70µg/ml among post-menopausal women and men, in the absence of other interfering variables. However, reports of competitive binding assays on MCF-7 cells estimated that the *o,p'*-DDT, *o,p'*-DDE, and *o,p'*-DDD (as well as *p,p'*-DDT, which did not bind to the rat receptor) hER affinity would be approximately 1,000-fold weaker than that of estradiol (Chen et al., 1997), i.e. about 100 times greater than with the E-screen assay. Besides, two yeast expression-reporter systems, constructed to test the ability of DDT isomers and metabolites to transactivate the hER, showed that the *o,p'*-DDE potency of transactivating the hER or LexA-hER fusion protein is 140-300-fold weaker than that of estradiol, and that DDT isomers and metabolites, as well as other xenoestrogen pesticides, elicited an additive response when given together or with estradiol (Chen et al., 1997; Soto et al., 1995). Based on such estimates, significant estrogenic effects would become manifest for *o,p'*-DDE blood concentrations of 4.2-54ng/ml (or ppm) in fertile women and 1.4-21ng/ml (or ppm) in post-menopausal women and men. With due caution for the interspecies differences, a study in rats, showing estrogenic effects at *o,p'*-DDE concentration above 18ng/ml (Ulrich et al., 2000), seems to be consistent with the *in vitro* based estimates of the order of magnitude of xenoestrogen blood level which would significantly interfere with natural estrogens.

The interference of xenoestrogens with the binding of sexual hormones to extracellular proteins, such as SHBG and α-fetoprotein, is another factor to be considered in estimating their effective estrogenic dose (Toppari et al., 1995). It is known that methoxychlor, *o,p'*-DDT, pentachlorophenol, and nonylphenol reduce [3H]17 β-estradiol binding to the estrogen receptor by 10, 60, 20, and 75%, respectively (Danzo, 1997), which would require proportionally higher doses of the respective pesticide to significantly compete with estradiol receptorial affinity. Therefore, it seems biologically implausible that blood concentrations of xenoestrogen pesticides below the ppm range could significantly interfere with the human endocrine system through a receptorial mechanism.

Other endocrine effects of pesticides

Pesticides may target also other endocrine glands. Toxaphene, for instance, cumulates in the rat adrenal cortex, where it inhibits ACTH-stimulated corticosterone synthesis (Kuz'minskaya & Ivanitskii, 1979; Mohammed et al., 1985). It also causes an increase in catecholamine breakdown, and unequal changes in the ratio of separate components of the sympathicoadrenalic system in tissues (Henderson et al., 1997). Besides, methyl parathion and malathion inhibit catecholamine secretion in bovine adrenal chromaffin cells (Liu et al., 1994). The pineal gland is another target of parathion toxicity, where it increased nocturnal N-acetyl transferase (NAT) activity and serum melatonin levels in rats, acting at the level of the beta-adrenergic receptor or via the sympathetic innervation to the pineal gland (Attia et al., 1991, 1995). An inhibitory effect of parathion on gonadotrophin secretion has been reported in Bobwhite quail (Rattner et al., 1982), and fish (Singh & Singh, 1981), but not in rats (Bentue-Ferrer et al., 1981).

Thyroid is another frequent target for endocrine effects of pesticides. Pesticides causing thyroid disruption in wildlife and experimental animals include DDT, polyhalogenated hydrocarbons – including toxaphene (Waritz et al., 1996), and methoxychlor (Zhou et al., 1995) –, phenol derivatives, amitrole, carbaryl (Ghosh et al., 1989), ethylenthiourea and other thiocarbamates (Brucker-Davis, 1998). In most instances, thyroid disruption results from an indirect effect, leading to an increase in Thyroid Stimulating Hormone (TSH) production. Extrathyroidal sites of action were found for amitrole, a widely used herbicide, ethylenethiourea (which also results from metabolism of numerous ethylenbisdithiocarbamate fungicides), and mancozeb, which are thyroid peroxidase inhibitors, and acetochlor, clofentezine, fenbuconazole, fipronil, pendimethalin, pentachloronitrobenzene, prodiamine, pyrimethanil, thiazopyr, and toxaphene which induce hepatic metabolism and excretion of thyroid hormones (Hurley, 1998; Waritz et al., 1996). Instead, methoxychlor would inhibit iodothyronine 5'-monodeiodinase, type I (5'-ID1), the enzyme that converts thyroxine to triiodothyronine (Zhou et al., 1995), and carbaryl caused a decrease in thyroxine level in the Channa punctatus fish, accompanied by a concurrent increase in acetylcholine accumulation and T3 level, due to a significant inhibition of brain acetylcholinesterase activity (Ghosh et al., 1989). However, typical environmental concentrations of potentially goitrogen chemicals did not significantly affect thyroid function among adult humans, while occupational or accidental exposure to higher levels produced only mild changes (Bogert et al., 1994).

Table 1

Estrogenic potential of some pesticides and therapeutic hormones with reference to estradiol, based on the in culture "E. Screen" assay (from Soto et al., 1995).

Compound	Concentration	RPE (%)	RPP (%)
17β-estradiol	10pM	100.00	100.00
DDT (technical grade)	10μM	79.61	0.0001
O,*p*'-DDT	10μM	86.14	0.0001
P,*p*'-DDT	10μM	71.00	0.0001
dieldrin	10μM	54.89	0.0001
Endosulfan (technical grade)	10μM	81.25	0.0001
1-hydroxychlordane	10μM	40.00	0.0001
Kepone	10μM	84.00	0.0001
Methoxychlor	10μM	57.00	0.0001
Toxaphene	10μM	51.90	0.0001

Table 2 summarizes results of *in vitro* studies, animal studies, and human studies for a list of pesticides which have been evaluated in relation to their potential endocrine disrupting effect. Negative reproductive outcomes due to teratogenicity or fetotoxicity are not included. The list is limited to the compounds for which information was available from the cited references and it is not meant to be exhaustive. It could be worthwhile to remind individuals that experimental data should be used to develop prudent guidelines and to identify reproductive and other endocrine hazards before human exposure occurs, but should not be considered as a proof of adverse effects in humans (Barrett, 1992).

Cancer risk associated with exposure to so-called endocrine-disrupting pesticides

Hormones play a major role in the etiology of several human cancers, including cancer of the breast, endometrium, prostate, ovary, thyroid, bone and testis (Bogert et al., 1994). Excessive hormonal stimulation of the normal growth and function of the particular target organ may result in neoplasia independent of outside initiators, such as chemicals or ionizing radiation. Also, hormone driven cell division allows the selective proliferation of the mutated clone resulting from the initial mutagenic event. Signif-

Table 2

Summary evaluation of pesticides with endocrine disrupting potential (EPA, 2000a, 2000b; EXTOXNET, 2000; Morgan, 1989; Tiemann et al., 1996; Toppari et al., 1995; Traina et al., 1994; Schrader & Cooke, 2000; Stelzer & Chan, 1999).

Pesticide	In-vitro studies	Animal studies	Human studies	Current uses
Organochlorines				
Acetochlor		Thyroid inhibitor		Herbicide in corn crops
Alachlor	Competitive aER and aPR binding	No reproductive effects in adult rats		Herbicide in corn crops, soybeans, peanuts, dry beans/peas, grain sorghum, and sunflowers
Chlordane and oxychlordane	No pro- or anti-androgenic effect	Reduced fertility in rats		Insecticide, termite control
Chlordecone (kepone)	Weak estrogen Competitive aPR binding	Reduced fertility	Reduction in sperm quality	Insecticide (tobacco, citrus trees, ornamental shrubs, bananas, ant & roach traps)
DDT congeners		Estrogenic effects thyroid inhibition	No evidence from population studies	Insecticide for Public Health purposes
o,p'-DDE	Estrogen			
p,p'-DDE	Anti-androgen			
Dieldrin	Weak estrogen Weak anti-androgen	No reproductive effects		Insecticide (corn and cotton crops)
Endosulfan	Weak estrogen. Competitive aER and aPR binding. Impaired steroid synthesis in Leydig cells	Damage to seminiferous tubules in male rats and reproductive organs in female mice		Insecticide (fruits, grains, tea, vegetables, tobacco, and cotton)
Heptachlor		Reduced fertility in rats		Insecticide, termite control
Methoxychlor	Estrogen	Testicular atrophy, decreased sperm production and testosterone levels in rats and mice. Reduced fertility in both genders. Thyroid inhibitor		Insecticide (fruits, vegetables, grain storage bins, mushroom houses, dairies, livestock)
Pentachlorophenol	Weak estrogen, weak anti-androgen	No reduced fertility in mice and rats		Biocide, wood preservative, pre-harvest defoliant in cotton
Hexachlorocyclohexane congeners		Reduced fertility	Changes in the levels of sex hormones.	insecticide (fruit and vegetable crops, including greenhouse vegetables, tobacco and forest crops), ointments to treat head and body lice, and scabies
α-HCH	Weak anti-androgen			
β-HCH	No effect	Weak estrogen-like effects in mice and rats		
γ-HCH (lindane)	Weak anti-androgen No estrogen effect Impaired steroid synthesis in Leydig cells	Testicular atrophy decreased sperm production and testosterone levels in rats and mink		
δ-HCH	Weak anti-androgen			
Mirex and photomirex	Weak estrogen No anti-androgenic effect	Reduced fertility due to testicular degeneration. Affects thyroid and parathyroid		Termiticide
Nonachlor (cis- and trans-)	Anti-estrogen Inhibits aER binding of [3H]17 β-estradiol	Sex-reversal in alligator embryos, and turtles		Termite control
Toxaphene	Weak estrogen. Inhibition of ACTH-stimulated corticosterone synthesis in the adrenal cortex	Thyroid inhibitor.		Insecticide, biocide

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Table 2 (continued from previous page)

Pesticide	In-vitro studies	Animal studies	Human studies	Current uses
Carbamates and thiocarbamates				
Aldicarb	Weak estrogen	No reproductive effects		Nematicide
Bendiocarb, methomyl, and oxamyl	Weak estrogens	No decrease if fertility in rats.		Insecticides, acaricides, nematicides
Carbaryl	Weak estrogen	Thyroid inhibition and reduced fertility in several animal species	Conflicting results on reduced fecundability	Insecticide
Carbofuran		Testicular damage in dogs		Broad spectrum insecticide
Mancozeb		Thyroid inhibitor in rats	Goitrogen	Fungicide
Maneb and Metiram		Thyroid inhibitors in several animal species. No reproductive effects		Fungicides
Molinate		Reduced fertility	No reproductive effects	Herbicide (rice crops)
Propoxur	Weak estrogen	Reduced fertility and lactation in female rats		Insecticide
Thiram		Infertility in male mice, delayed estrous cycle in female mice		Fungicide, animal repellent
Zineb		Thyroid inhibitor in several animal species. Reduced fertility in rats.		Fungicides
Ziram		Reduced fertility in female rats and mice and in male rats. Testicular atrophy		Fungicide on almonds and stone fruit
Organophosphates				
Chlorpyrifos		No reproductive effects		Insecticide and acaricide for crops and livestock
Dimethoate		No reproductive effects	Reduced fecundability	Insecticide for 40 different crops, lawns, termiticide in buildings, pet collars.
Methamidophos		Reduced number of deliveries in female rats	Reduced sperm count and viability	Insecticide, acaricide and avicide
Methyl parathion and malathion	Inhibits catecholamine secretion	No reproductive effects in rats		Insecticide for various crops, livestock, parasite control.
Oxydemeton-methyl		No reproductive effects in rats		Insecticide for various crops
Parathion		Increased nocturnal synthesis of melatonin. Gonadotrophic hormone inhibition		Insecticides for various crops
Trichlorfon		Reduced fertility and increased embryonic deaths in rats.		Insecticide for various crops, livestock, parasite control

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Table 2 (continued from previous page)

Pesticide	In-vitro studies	Animal studies	Human studies	Current uses
Other insecticides				
Amitraz		Decreased fertility in rats.		Insecticide (cotton and animals)
Amitrole		Thyroid inhibitor in several animal species.		Herbicide
Atrazine	Weak estrogen Weak anti-androgen Inhibits aER binding of [3H]17 β-estradiol	Damage to adrenal glands Impairment of steroid hormone metabolism		Herbicide (rice and other crops)
Azadirachtin		Infertility in several animal species		Insecticide
Benomyl (and its breakdown product carbendazim)	Microtubule disruptor	Decreased sperm production in adult male rats		Fungicide (field crops, fruits, nuts, ornamentals, mushrooms, and turf)
Captan		Fetal loss or reduced weight at birth in mice.	Reduced fecundability	Fungicide (apple production, ornamental and vegetable crops)
Clofentezine		Thyroid disruptor. No reproductive effects		Acaricide
2,4-D		No reduction in fertility. Increase in thyroid and ovarian weights.	Reduced fecundability	Herbicide (wheat, rice and other crops, lawn, pasture, home, garden)
Dibromochloropropane		Infertility in rabbits (atrophy of testes)	Infertility. Potent testicular toxicant	Insecticide (banana plantations)
Dicamba		No reduction in fertility in rats and rabbits.	Reduced fecundability	Herbicide (pastures, non crop areas, roadways to control weeds)
Dinoseb		Reproductive effects in rats		Herbicide in various field crops; insecticide in grapes
Ethylene dibromide		Reduced sperm count in bulls	Reduced sperm count and quality	Post harvest fumigant
Fenbuconazole		Thyroid inhibitor		Fungicide
Fipronil		Thyroid disruptor Reproductive toxicant in rats		Insecticide
Glyphosate		Reproductive changes at very high doses	Reduced fecundability	Herbicide
Hexachlorobenzene		Decreased male fertility. No estrogenic effects		Fungicide used to dress seed and treat soil. Contaminant of PCNB
Hexazinone		No effects on reproduction and lactation in rats		Herbicide in non-crop areas and weed control for sugarcane and pineapples
Linuron		No reproductive effects		Herbicide (soybean, cotton, potato, corn, carrots, celery, sorghum, and asparagus)
Metolachlor		Testicular atrophy in rats. No reproductive effects in mice		Herbicide
Metribuzin		No reproductive effects. Thyroid enlargement in male rats.		Herbicide

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Table 2 (continued from previous page)

Pesticide	In-vitro studies	Animal studies	Human studies	Current uses
Nonyl-phenol	Weak estrogen			Molluscicide
Pentachloronitrobenzene (PCNB)		Thyroid inhibitor		Fungicide used to dress seed and treat soil
Propiconazole		No reproductive effects in rats		Fungicide on cereals, fruit and green forage crops
Procymidone		Anti-androgen in rats		Fungicide
Prodiamine		Thyroid disruptor		Herbicide
Pyrimethanil		Thyroid inhibitor No reproductive effects		Fungicide
Simazine		No reproductive effects in rats. Distrophy and necrosis of germ cells in sheep.		Herbicide, submerged weeds and algae control in large aquariums, ponds and fish hatcheries.
Thiazopyr		Thyroid disruptor No reproductive effects		Herbicide
Tributyltin		Short-term decrease in the activity of the pituitary-thyroid axis in rats. No long-term effects. Pseudoermaphroditism in gastropods,		Antifouling agent on ships, boats, and mariculture pen nets
Vinclozolin	Anti-androgen	Demasculinizing effects in rats. No reproductive effects		Fungicide (grapes, vegetables, strawberries, fruit, ornamentals)

icant evidence exists that certain estrogens can also cause genetic alterations by mechanisms that do not involve the classical estrogen receptor (Bogert et al., 1994). The absolute frequency of these cancers is a reason for concern about cancer risk associated with medical treatment or occupational and environmental exposure to the same or closely related hormones. Experimental animal studies indicate that some estrogen mimicking pesticides might produce the same carcinogenic effects as natural or therapeutic estrogens. For instance, estrogen-like effects of methoxychlor caused a dose-related atrophy of the testes in rabbits and testicular cancer in BALB/c mice, and cancer of the ovary, pituitary, adrenals, and mammary gland in Osborne-Mendel female rats (Reuber, 1979, 1980). Hyperplasia of the mammary gland and uterus was also observed in miniature swine (Reuber, 1980). On the other hand, oral administration of DDT did not induce testicular cancer in BALB/c and C3H mice (Reuber, 1979), and inter-species differences in susceptibility to the hormonal and carcinogenic effects of methoxychlor were reported (Reuber, 1980).

Experimental evidence exists that hormone-like pesticides may stimulate cell proliferation and tumorigenesis also through mechanisms other than the classical hormonal pathways. For instance, *p,p'*-DDT, but not *o,p'*-DDT, elevated tyrosine phosphorylation in c-erbB2and c-met growth factor receptors, the STAT1 alpha (p84/91) signal transduction indicator, and 3H thymidine incorporation in human breast epithelial MCF-10A cells at physiologically relevant concentrations, such as 10nM (Shen & Novak, 1997). However, only cancer sites for which hormonal mechanisms have been postulated are herein considered.

Breast cancer

The role of hormones in breast cancer etiology is consistent with the hypothesis that breast cell proliferation is estrogen-dependent (Bogert et al., 1994). Polymorphisms of genes involved in estrogen secretion and metabolism, such as:

a) the 17B-hydroxysteroid dehydrogenase 2 (EDH17B2) gene encoding the 17B-hydroxysteroid dehydrogenase enzyme, which converts estrone (E1) into estradiol (E2);

b) the cytochrome P450c17a (CYP17) gene, which controls certain rate limiting steps in estrogen biosynthesis; and

c) the estrogen receptor gene, encoding the estrogen receptor which binds and transactivates estrogen into DNA for transactivation of estrogen responsive genes, might account for

geographic variation in breast cancer incidence. All these metabolic steps may be also affected by xenobiotics, thus leading to an excess or a decrease in estrogen stimulation of the target breast cells. These indirect effects may contribute to, or may decrease the effect of direct receptorial stimulation.

A population-based case control study in British Columbia reported an excess breast cancer risk among women (pre- and postmenopausal ages combined) employed as crop and fruit and vegetable farmers, with a reasonably likely exposure to numerous pesticides (Band et al., 2000). Occupational exposure to pesticides was also significantly associated with a 4-fold increase in breast cancer risk in Serbia (Kocic et al., 1996), and risk was also elevated among Chinese women with some probability of high level pesticide exposure, although this finding was based on a small number of exposed (Petralia et al., 1998). Female breast cancer mortality and incidence was not increased among Florida licensed pesticide users (Fleming et al., 1999a, 1999b), while a case-control study on occupational risk of male breast cancer did not find an association with exposure to herbicides and other pesticides (Cocco et al., 1998). Also, agricultural occupations were not included among high risk occupations for breast cancer in Swedish women (Pollan & Gustavsson, 1999). In most of these studies, exposure to pesticides was largely hypothetical, based on occupational titles or derived from working histories. Studies measuring pesticide residuals in body fluids or tissues as markers of internal dose of exposure are supposed to provide a more precise definition of exposure. Besides, as stated above, exposure to pesticide mix, resulting from numerous different chemicals, which change rapidly on a yearly base, is the norm in agricultural settings, making it difficult to identify the potential risk factor. On the other hand, by definition, studies using markers of internal dose are aimed to identify associations with specific compounds or their derivatives, and only chemicals for which specific tests are available may be identified. Therefore, it is uncertain how to interpret contradictory findings, whether in relation to random variation in the response to the known chemical, or to changes in the associated chemicals, or factors responsible for the increase in risk. Most such reports focused on the association between organochlorines, particularly DDT derivatives, and breast cancer. A large number of such studies have been published, on the wave of few positive reports. Table 3 summarizes their results.

A vast amount of scientific effort and resources have been dedicated to explore the risk of breast cancer associated with the internal dose of DDT derivatives. Among the 29 studies reported in Table 2, the majority (21/29) yielded negative results, and only 5/29 found a significant excess risk, ranging 2-5 fold (a ninefold increase was reported in a small study for ER+ mammary cancer – Dewailly et al., 1994). A logical conclusion would be that there is not epidemiological evidence that the internal dose of DDT derivatives, mostly *p,p'*-DDE, is associated with an increase in breast cancer risk. Such a conclusion fits with the current experimental knowledge about *p,p'*-DDE as a potent anti-androgen (Kelce et al., 1995). The estrogen derivative, *o,p'*-DDE, derives from *o,p'*-DDT, which is only a contaminant of technical grade DDT (IARC, 1991). *o,p'*-DDT concentration may vary according to the producer, but is usually much lower than the *p,p'* isomer. As a consequence, *o,p'*-DDE is frequently below the limit of detection in population studies, which would exclude a significant contribution in increasing breast cancer risk. On the other hand, we cannot exclude a possible role of *o,p'*-DDE in breast cancer etiology, if such exposure would occur at a high level.

The body burden of other organochlorines has been measured in relation to breast cancer risk. High dieldrin serum concentrations were associated not only with a 2-fold increase in breast cancer risk (Wolff et al., 2000b), but also with poor prognosis among breast cancer patients (Hoyer et al., 2000a). Findings were less suggestive for hexachlorobenzene (HCB). HCB breast adipose tissue levels did not differ significantly between incident breast cancer cases and non cancer controls (Aronson et al., 2000; Dewailly et al., 1994; Falck Jr. et al., 1992; Zheng et al., 1999a), although they were slightly more elevated among ER+ breast cancer cases compared to ER- cases, and to controls (Dewailly et al., 1994; Zheng et al., 1999b). A non significantly elevated risk was found only among nulliparous women, for the third tertile when compared with the lowest. No excess was observed among parous women, or for premenopausal or postmenopausal breast cancer (Zheng et al., 1999b). In another study, women in the upper three quartiles of HCB serum level were at twice the risk of breast cancer, compared to those in the lowest quartile (Dorgan et al., 1999). However, there was no evidence for a dose-response relationship, and the association was limited to women whose blood was collected close to the time of diagnosis. In a third study, only postmenopausal women

Table 3

Studies of breast cancer in relation to biomarkers of internal DDT exposure.

First author and year	Body fluid/tissue	# of cases and controls	Country	DDE concentration (cases *vs* controls)	Risk
Wassermann et al. (1976)	Mammary adipose tissue	9/5	Brazil	2.0 *vs* 4.3ppm	Not evaluated
Unger et al. (1984)	Mammary adipose tissue	14/21	Denmark	1.23 *vs* 1.25ppm	No association
Mussalo-Rauhamaa et al. (1990)	Mammary adipose tissue	41/33	Finland	960 *vs* 980ppb	No association
Falck Jr. et al. (1992)	Mammary adipose tissue	20/20	United States	1887 *vs* 1174ppb	Non significant excess
Wolff et al. (1993)	Serum	58/171	United States	11.0 *vs* 7.7ng/ml	4-fold excess
Dewailly et al. (1994)	Mammary adipose tissue	20/17	Canada	2131.2ppb (ER + cases) *vs* 608.9ppb (ER - cases) *vs* 765.3ppb (controls)	9-fold excess (ER + cases)
Krieger et al. (1994)	Serum	150/150	United States	43.3 *vs* 43.1ppb	No association
Van't Veer et al. (1997)	Adipose tissue	265/341	Europe	1.35µg/g *vs* 1.51µg/g	Significant decrease
López-Carrillo et al. (1997)	Serum lipids	141/141	Mexico	562.5 *vs* 505.5ppb	No association
Hunter et al. (1997)	Plasma	236/236	United States	4.71 *vs* 5.35ppb	Non significant decrease
Schecter et al. (1998)	Serum	21/21	Vietnam	12.17 *vs* 16.67ng/ml	No association
Moysich et al. (1998)	Serum lipids	154/192	United States	11.47 *vs* 10.77ng/ml	Non significant excess
Guttes et al. (1998)	Breast tissue	45/20	Germany	805 *vs* 496ng/g	Not evaluated
Liljegren et al. (1998)	Adipose tissue	43/35	Sweden	767 *vs* 1,026ng/g	No association
Olaya-Contreras et al. (1998)	Plasma	153/153	Colombia	3.3 *vs* 2.5ppb	2-fold excess
Hoyer et al. (1998)	Serum lipids	240/477	Denmark	1,168.2 *vs* 1,185.4ppb	No association
Dorgan et al. (1999)	Serum	105/525	United States	Controls:16.3ng/ml Cases: lower but not given	No association
Helzlsouer et al. (1999)	Serum lipids	346/346	United States	1,698.9 *vs* 1,920.3ng/g	Inverse association
Mendonça et al. (1999)	Serum	177/350	Brazil	5.1 *vs* 4.8ng/ml	No association
Zheng (1999b)	Adipose tissue	304/186	United States	736.5 *vs* 784.1ppb	No association
Aronson et al. (2000)	Adipose tissue	217/213	Canada	693 *vs* 596µg/Kg	No association
Bagga et al. (2000)	Adipose tissue	73/73	United States	800.0 *vs* 709.1ng/g	No association
Demers et al. (2000)	Plasma lipids	315/526	Canada	508.9 *vs* 426.7 (Hosp. Ctrls) and 480.4µg/kg (Pop. Ctrls)	37% excess with Hosp Ctrls. 64% excess for large tumors. 2.9-fold excess for lymphnode involvement
Hoyer et al. (2000b)	Serum lipids	155/274	Denmark	1,229.8 *vs* 1,171.6ppb	3-fold excess for p,p'-DDT no excess for p,p'-DDE
Romieu et al. (2000)	Serum lipids	120/126	Mexico	3.84 *vs* 2.51µg/g	Postmenopausal: 5-fold excess. Premenopausal: 2-fold excess.
Wolff et al. (2000a)	Serum lipids	175/355	United States	0.61 *vs* 0.66µg/g	No association
Wolff et al. (2000b)	Serum lipids	148/295	United States	977 *vs* 1\100ng/g	No association
Zheng et al. (2000)	Serum	475/502	United States	460.1 *vs* 456.2ppb	No association

showed a non significant increase in breast cancer risk associated with HCB fat concentrations around 40ng/g, but postmenopausal women with ER+ tumours showed a 7-fold increase in risk (Liljengran et al., 1998). On the other hand, limited evidence of an adverse effect of serum levels of HCB and mirex, another chlorinated insecticide, only for women who ever lactated was observed in another U. S. study (Moysich et al., 1998). Higher β-Hexachlorocyclohexane (HCH) plasma levels were associated with an increase in breast cancer risk in three studies (Aronson et al., 2000; Hoyer et al., 1998; Mussalo-Rauhamaa et al., 1990). However, lower β-HCH concentrations were reported in the breast adipose tissue of cancer patients than in tissue from women with benign disease in a German study (Guttes et al., 1998). Other pesticides were evaluated in individual studies. A Canadian study reported positive findings for oxychlordane and transnonachlor (Demers et al., 2000), while triazine

herbicides showed a positive association with breast cancer incidence in an ecological study among Kentucky counties in the United States (Kettles et al., 1997).

Only DDT derivatives have been extensively investigated thus far. In the overall evaluation, results have been negative. However, studies should address more specifically the hypothesis on whether a receptorial and/or non receptorial mechanism is implicated. If a receptorial mechanism is hypothesised, the most estrogenically effective pesticides should be investigated, including the *o,p'*- DDT isomer and its derivative *o,p'*-DDE, as well as other pesticides with significant estrogenic power, particularly in relation to ER+ neoplasms in the breast and other sites as well.

Endometrial cancer

The major demographic characteristics of endometrial cancer, as well as the major non demographic risk factors, are explained on the basis of cumulative exposure of the endometrium to the so-called unopposed estrogen fraction, which is not counterbalanced by the modifying influences of progesterone. Low parity is the major established risk factor for endometrial cancer, as no endometrial mitotic activity occurs during pregnancy, due to the persistent high progesterone levels. Oral contraceptives, that expose the endometrium to constant high levels of both estrogen and progestogen, should also protect against endometrial cancer development, while estrogen replacement therapy and obesity should increase risk. All of these predicted effects have been repeatedly well documented in epidemiological studies (Bogert et al., 1994). Obese postmenopausal women have increased plasma concentration of estradiol, as adipose tissue converts androstenedione to estrone, which in turn can be converted directly to estradiol. In addition, SHBG levels are lower in obese women, so that their amount of bioavailable estradiol is higher than would be expected from the peripheral conversion of androstenedione to estrone alone (Bogert et al., 1994). Therefore, also xenoestrogens might add their effect to natural estrogen playing a role in the development of endometrial cancer.

Only a few studies, however, have specifically addressed the issue. No excess in endometrial cancer risk associated to increasing quartiles of serum concentrations of *p,p'*-DDE was observed in a multicentre case-control study conducted in five geographic regions of the United States (Sturgeon et al., 1998). Also, blood concentration of various chlorinated pesticides was not associated with an increase in endometrial cancer risk (Weiderpass et al., 2000), or with diagnosis of endometriosis (Lebel et al., 1998). Endometriosis is a relatively frequent disorder characterized by the growth of endometrial tissue in abnormal locations, most frequently in the ovary (Perloe, 2000), which is associated with an increased ovarian cancer risk (Ness et al., 2000).

Ovarian cancer

It is proposed that the epithelial cells within the developing follicle or covering the ovarian surface, which replicate during or after each ovulation, are the cells of origin for ovarian cancer (Bogert et al., 1994). Thus, any respite from ovulation would be protective against ovarian cancer, as supported by epidemiological results showing that the ovarian cancer risk decreases with increasing parity, combination oral contraceptive use (Bogert et al., 1994), and lactation, at least for ovarian cancers occurring in the premenopausal age (Daly & Obrams, 1998; Riman et al., 1998; Siskind et al., 1997). Triazine herbicides, which have shown weak estrogen and anti-androgen effects in *in vitro* studies (Table 1), have been associated to a 2.7-4.4-fold increase in ovarian epithelial cancer in two Italian case-control studies (Donna et al., 1984, 1989). These results need to be replicated and the carcinogenic mechanism elucidated before drawing conclusions.

Prostate cancer

Although the relationship between testosterone levels and the rate of cell proliferation in the prostate is unstudied, and epidemiological studies of circulating testosterone levels and prostate cancer risk yielded conflicting results, it is well known that its metabolic product, dihydrotestosterone, controls mitotic activity in the prostate, by binding to the androgen receptor (AR) and being then translocated to the nucleus of prostate cells for DNA binding and transactivation of androgen responsive genes (Bogert et al., 1994). All clearly established experimental models of prostate cancer have an androgen requirement for tumor induction. The AR, which is the product of the AR gene located on the long arm of the X chromosome, is crucial for androgen activity in the prostate. The frequency of AR gene polymorphisms, that transactivate subnormally an androgen responsive reporter gene, vary between populations consistently to vari-

ations in prostate cancer incidence (Bogert et al., 1994).

Occupational and environmental exposure to pesticides potentially interacting with free testosterone bioavailability, and/or its AR binding, might be relevant for prostate cancer risk. Such exposures are more likely to occur among agricultural workers or for the general population living in rural areas, or around chemical factories, where pesticides are used, manufactured and/or processed. Reviews of the epidemiological literature on the occupational risk for prostate cancer have indicated an excess among farmers (Blair et al., 1985; Keller-Byrne et al., 1997; van der Gulden & Vogelzang, 1996). A meta-analysis of 24 studies which examined the association of prostate cancer with farming found a weak positive association overall. The observed excess resulted mainly from retrospective case-control studies, it was not affected by the year of publication, and it was not explained by the possible confounders (Keller-Byrne et al., 1997). The interpretation to be given to these findings also varies by the attitude of the author, and the use of agricultural chemicals is among the considered hypotheses (Keller-Byrne et al., 1997; van der Gulden & Vogelzang, 1996). More recent reports have not provided more consistent findings. A two-fold excess prostate cancer risk among United States farmers was restricted to short-term workers and workers in crop production, and it was not limited to those who began farming after the widespread introduction of pesticides use (Krstev et al., 1998). Dutch farm laborers who worked between 1960 and 1970 had a significant increase in prostate cancer risk, and they significantly differed from workers who had controls for spraying pesticides more days per year (van der Gulden et al., 1995). Farming employment was the most prominent positive association in another United States case-control study, where patients affected by benign prostate hyperplasia were the controls (Checkoway et al., 1987), and in an occupational survey in British Columbia, using cancer patients (all other sites but lung) as the controls (Band et al., 1999). However, although exposure to herbicide and pesticides was more common among cancer patients, ages when starting farm work, years worked, hours of farm work per week, and proportion of exposed to pesticides and herbicides did not differ between cases and controls who reported ever being employed on a farm. Therefore, the authors concluded that occupational exposures in farming did not account for the observed association (Checkoway et al., 1987). In a Canadian study, more detailed occupational information suggested a weak, but significant excessive prostate cancer mortality with the number of acres sprayed with herbicides in the 1970's, while no other farm exposures evaluated in this study was associated with an increase in prostate cancer risk (Morrison et al., 1993). In a German case-control study of 192 histologically confirmed prostate cancer cases, however, the only reported occupational risk factor was having worked in transportation or communication activities (Heiskel et al., 1998). It is not clear from this small study whether driving was the risk factor. If it were, the hypothesis should be considered also among farmers, as driving tractors and heavy duty agricultural machines in unpaved country roads is an important part of agricultural work, and therefore a possible confounder in the studies of pesticide use and prostate cancer.

Cohort studies of pesticide applicators show somewhat more consistent positive findings, with a two-fold increase in mortality from (Fleming et al., 1999a) and the incidence of prostate cancer (Fleming et al., 1999b) in Florida. A 26% excess in a proportional mortality study of Iowa farmers (Cerhan et al., 1998), and a small but significant 13% excess incidence in a large Swedish cohort of licensed pesticide applicators in agriculture, which was more elevated among those born in 1935 or later. In the opinion of the authors, this would corroborate the hypothesis of a link with agrochemicals or other risk factors in farming (Dich & Wiklund, 1998).

In contrast with occupational studies, where heterogeneous exposures are accumulated into definitions such as "farmers", "pesticides" or – at best – "herbicides", some environmental epidemiology studies evaluating hormone-related cancer risk focused on specific chemicals. On the other hand, conducting studies at the population level exposes the effect of the so-called "ecological bias" (Morgenstern, 1982). Such a bias results in a dilution of the risk estimate in case of a true positive association, as there is no way of distinguishing within a general population various degrees of exposure, if they are not related to physical and measurable entities, such as distance from the source. Although in a study of 24 non human primates, fed with 20mg/Kg DDT daily for more than 10 years, one out of the two tumors observed was a well-differentiated adenocarcinoma of the prostate (Takayama et al., 1999), environmental exposure to DDE, the most persistent DDT derivative, as derived from early measurements in the adipose tissue of population samples in

22 states of the United States, did not show a positive correlation with prostate cancer mortality (Cocco & Benichou, 1998). In fact, those results might suggest a negative correlation if any, a paradox which could be biologically plausible, due to the anti-androgenic properties of the main DDE isomer, the *p,p'* isomer, in experimental models (Kelce et al., 1995). Pounds of atrazine and captan applied annually in Central California counties showed a good correlation ($r = 0.67$ and 0.49, respectively) with incidence of prostate cancer (Mills, 1998). A small increase in prostate cancer mortality was observed in the surroundings of a pesticide factory in United Kingdom, but a link was excluded as there was not an indication of a decline with distance from the factory (Wilkinson et al., 1997). 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) is a frequent contaminant of widely used herbicides. TCDD-related anti-estrogenic effects have been described in experimental animals as well as in MCF-7 human breast cancer cell cultures (Safe et al., 1991). As estrogen-treatment of prostate neoplasms is a therapeutic strategy, a possible link of exposure to anti-estrogen herbicides and prostate cancer risk was enviseged (Keller-Byrne et al., 1997). However, the 15-year mortality experience among the population of the TCDD-contaminated area after the Seveso accident in Italy, did not show any increase in prostate cancer deaths (Bertazzi et al., 1997), while the complex interaction of TCDD with the endocrine system, along with its cardiovascular and immune toxicity, was tentatively considered as a plausible explanation for the excess mortality from cardiovascular diseases and diabetes in this population (Pesatori et al., 1998).

It seems plausible that occupational exposure to very high concentrations of some pesticides, particularly those which bio-concentrate in fat, might affect prostate cancer risk (Keller-Byrne et al., 1997). However, assuming exposure to pesticide concentrations high enough to compete with natural hormones in linking specific receptors and disrupt significantly their effect, it is also plausible that an increase or decrease in risk might result; depending on the specific type of hormone-like mechanism involved. Therefore, more research should be devoted to inquiry into specific chemicals, or well defined mixtures, and specific toxicological mechanisms affecting prostate cancer risk.

Testicular cancer

Some epidemiological characteristics of testicular cancer suggest a role for *in utero* estrogen exposure in the etiology of this tumor. Apart from the characteristic peak incidence rate in early adult life, in a pattern that is similar to that of DES-induced vaginal adenocarcinoma, cryptorchidism, maternal nausea and maternal obesity, both related to excess free maternal estrogen during the critical gestation period, these are known risk factors for testicular cancer (Bogert et al., 1994). Such a hypothesis was confirmed in a recent large case-control study in which xenoestrogen exposure during pregnancy was associated with a 4-fold increase in the risk of primary malignant germ-cell testicular cancer among residents in Ontario aged 16-59 (Weir et al., 2000). The hypothesis that parental exposure to estrogen-mimicking pesticides could play a role in testicular cancer etiology among youngsters was tested in a large cohort study of children born from 1952-1991 to Norwegian farmers (Kristensen et al., 1996). However, while a higher than expected incidence of testicular cancer was observed, the excess was related to use of fertilizers, particularly for non-seminoma neoplasms. Therefore, the authors raised the hypothesis of a role of excess run-off nutrients from agricultural areas instead of endocrine disruption as the underlying mechanism (Kristensen et al., 1996). Some indication of a species-specific response of the Leydig cells of the testicular interstitium to the stimulation of the luteinizing hormone (LH), following administration of the fungicide procymidone, is provided by experimental studies in rats and mice (Murakami et al., 1995). This could account for the different sensitivity across species to the procymidone-induced Leydig cell tumorigenesis. Within species differences might be related to genetic polimorphisms, such as the overexpression of aromatase, which might result in itself the increased estrogen production, leading to the induction of testicular cancer (Fowler et al., 2000).

The finding of an excessive risk of testicular seminoma and testicular dysfunction among United States military dogs serving in Vietnam, coupled with a decrease in sperm count among United States Vietnam veterans, suggested a role of phenoxy herbicides in testicular cancer etiology (Hayes et al., 1990). On the other hand, a 2.5-fold increase in testicular cancer incidence was reported among Florida licensed pesticide applicators, but, although this result would add to the evidence supporting a role of endocrine disrupting pesticides, the lack of soft tissue sarcomas in this cohort was considered at odds with a role of phenoxy herbicides (Fleming et al., 1999b). Also, an elevated risk of testicular cancer among Swedish licensed

pesticide applicators, reported in an early paper (Wiklund et al., 1986), was not confirmed in the extended follow-up (Dich et al., 1996). Geographical studies provided limited support for an excess of surgical correction of cryptorchidims (orchidopexy), a condition strongly associated with testicular cancer risk, in Spanish areas with more intensive farming (Garcia-Rodriguez et al., 1996). No specific agricultural chemicals have been investigated in relation to testicular cancer risk, using an analytical study design. An ecological study of testicular cancer mortality in relation to DDE body burden in population samples of 22 states of the United States (Cocco & Benichou, 1998) did not support the hypothesis of an involvement of this chemical in causing the repeatedly reported rising incidence of this tumor (Ekbom et al., 1996; Skakkebaek et al., 1998).

Thyroid cancer

As TSH is the principal hormone regulating the growth and function of the thyroid gland, any mechanism by which elevated TSH levels are achieved may be of etiologic relevance in the development of thyroid cancer (Bogert et al., 1994). For instance, history of pregnancy, during which thyroid gland activity is increased, is associated with elevated thyroid cancer risk. Other therapeutic or environmental conditions, such as blocking thyroid hormone synthesis, administering TSH directly, an iodine-deficient diet, or environmental exposure to chemical goitrogens, including a few pesticides, may also affect the thyroid function thus contributing to an increase in thyroid cancer risk (Bogert et al., 1994). Thyroid is a frequent target for carcinogenic pesticides in experimental studies. Ten percent of pesticides screened for carcinogenicity by the Environmental Protection Agency, United States (EPA, 2000a, 2000b), produced thyroid follicular cell tumors in rodents, but mutagenicity seemed to be relevant only for acetochlor (Hurley, 1998; Mattioli et al., 1994), and amitrole. Amitrole is metabolized to mutagenic intermediates by peroxidases, including prostaglandin synthetase, and lactoperoxidase, a model for thyroid peroxidase (Bogert et al., 1994). Epigenetic mechanisms are suspected as intrathyroidal and extra-thyroidal sites of action were reported for numerous pesticides which induce hepatic metabolism and excretion of thyroid hormones (Hurley, 1998).

Conclusions

In conclusion, scientific evidence exists that DCMP, chlordecone, and maybe a few other pesticides, affect human reproduction, while amitrole and mancozeb are thyroid inhibitors. As it concerns other pesticides, results are either negative, or they are contradictory, or, when positive, they are observed only at very high doses in experimental animals, or simply they have not been studied. Whether human exposure to the broad category of pesticides should be regarded as a risk factor for hormone-dependent cancers and endocrine disruptive effects is still unclear. In fact, there is not conclusive evidence for such effects following occupational and environmental exposure to pesticides, as contradictory findings reflect the extraordinary heterogeneity of exposures within the category. On the other hand, there is evidence of an adverse effect of some pesticides on the human endocrine system, while others require further investigation for a possible etiologic role in some hormone-dependent cancers. From the perspective of prevention, to keep using the broad definition of pesticides in epidemiological studies does not help to identify the responsible agent. Indeed, the complexity of exposures results in dilution of effects, as most individual compounds are not studied in this regard, most might be ineffective as endocrine disruptors or carcinogenic agents, or there might be opposite effects. Also, apart from receptorial mechanisms, other non-receptorial mechanisms may plausibly play a role in endocrine disruption and hormone-dependent cancer, and individual chemicals should be evaluated in this regard.

As far as specific chemicals are concerned, DDT is the most studied chemical in this respect. This chemical was banned almost 30 years ago in developed countries, and it is currently used only in developing countries against malaria, because it is still effective and inexpensive. While a precautional world-wide ban of its agricultural uses is reasonable, banning its use for Public Health purposes is not, as the certainty of its anti malarial effectiveness is not balanced by the uncertainty of the resulting benefits. Indeed, it seems contradictory to attribute to this compound, whose body burden in humans is steadily decreasing, the responsibility of a hypothetical parallel world-wide reduction in male fertility or increase in testicular cancer incidence (Cocco, 1997; Ekbom et al., 1996; Safe, 2000).

Experimental and animal studies underscore an ecological problem, which is a serious

one in itself, as well as a reason for concern, restriction, and control of the proper use of such chemicals. Studies in wildlife, however, seldom correspond to the standards required to the epidemiological and experimental work in terms of study design and analysis, which allow researchers to test the same hypothesis under the same or similar conditions before establishing conclusive remarks. More sound scientific work is needed to prevent ecological and human adverse effects, including endocrine effects, resulting from improper use of pesticides or from proper use of hazardous chemicals.

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