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Influence of pesticides on male fertility

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Several studies have shown a decline in human semen quality and increased risks of male subfertility. This paper provides an overview of the mechanisms of pesticide-induced reproductive toxicity and the effects on male fertility since exposure to pesticides may be one of the causes of these disorders. Pesticides may directly damage spermatozoa, alter Sertoli cell or Leydig cell function, or disrupt the endocrine function in any stage of hormonal regulation (hormone synthesis, release, storage, transport, and clearance; receptor recognition and binding; thyroid function; and the central nervous system). These mechanisms are described with respect to the effects of pesticide exposure in vitro and in vivo. In epidemiologic studies, effects on sperm quality and time to pregnancy are reviewed. Clear effects on male fertility have been demonstrated for some pesticides [eg, dibromochloropropane, ethylene dibromide]. But results from more recent studies are inconsistent, and no uniform conclusion can be drawn about the effects of pesticides on male reproduction.

Key terms endocrine disrupting chemical; hormone regulation; overview; sperm quality; time to pregnancy.

The male testes fulfill two essential functions, spermatogenesis in the seminiferous tubules, producing male gametes, and synthesis and secretion of sexual hormones in the interstitium. The production of sexual hormones is regulated by the hypothalamus-pituitary-gonadal axis, comprising the hormones gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and inhibin B (1). LH and FSH are produced by the pituitary gland under the influence of pulsatile secretions of GnRH released by the hypothalamus. LH stimulates Leydig cells in the testes to produce testosterone, which is an important hormone for spermatogenesis through the stimulation of Sertoli cells in the seminiferous tubules. The main function of Sertoli cells is to create a favorable environment for germ cell proliferation and maturation. FSH controls spermatogenesis via direct stimulation of Sertoli cells. It also stimulates inhibin B synthesis in the Sertoli cells. Both testosterone and inhibin B regulate GnRH and LH or FSH secretion through a negative feedback loop. For normal spermatogenesis to occur, adequate functioning of this endocrine regulatory system and the two testicular compartments is necessary (2).

In the complex regulation of hormone production and spermatogenesis, disturbances can easily occur and result in diminished or absent spermatogenesis and thus

sub- or infertility. Subfertility is defined as the inability to conceive after 12 months of regular, unprotected sexual intercourse, and it affects approximately 15% of all couples in the Western world (3–6). A multi-centre study of the World Health Organization (WHO) revealed that the problem was predominantly male in 20% of subfertile couples and predominantly female in 38% of the cases, whereas 27% showed abnormalities for both men and women, and no evident cause of subfertility was identified for the remaining 15% (7). In 98% of male subfertility cases, deficient spermatozoal quality is the main cause, and for the remaining 2%, inadequate sexual or ejaculatory function is the cause of subfertility (8). Male subfertility can be categorized as being due to pretesticular, testicular, or post-testicular factors, which are listed in appendix 1 (7, 9). For most men, however, subfertility is regarded as idiopathic (7) and can be due to several exogenous risk factors, such as smoking, alcohol, and coffee consumption and environmental endocrine disruptors. In the past two to three decades, it has become clear that occupational exposure to specific pesticides may also affect the fertility of men (10–12).

Therefore, in this review, we focus on the effects of pesticides on male reproduction. First, we give an overview of the possible mechanisms through which pesticides can affect male fertility. Thereafter, the evidence

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for effects of occupational exposure to pesticides on semen quality and time to pregnancy are evaluated.

Methods

A thorough literature search of articles in English and Dutch published between 1970 and July 2005 was performed using MEDLINE, bibliographies of published works, and textbooks. The search terms used were pesticides, insecticides, fungicides, herbicides, hormone disrupting chemicals, and endocrine disruptors. These search terms were combined with terms such as hormone metabolism, FSH, LH, testosterone, spermatogenesis, sperm concentration, time to pregnancy, and fertility. In the section of the paper describing the mechanisms of pesticide action, we primarily used studies involving laboratory animals (in vivo) or cell cultures (in vitro). In the section on the effects of pesticides, our focus was on literature discussing the influence of pesticide exposure on adult human males. No quality criteria were used for the inclusion of published papers in the overview, but remarks about potential shortcomings of several studies have been made in the text and tables.

Overview of the literature

Mechanisms of pesticide-induced reproductive toxicity

Pesticides are used in agriculture and public health to control insects, weeds, animals, and vectors of disease. In a revised version of the International Code of Conduct on the Distribution and Use of Pesticides, the definition of pesticides is "any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies [p 6]" (13). Next to these useful properties, pesticides can affect human health, including male reproductive function.

Damage to the testes can involve all of the cell types mentioned earlier. Damage to the spermatozoa or their precursors can result in reversible or irreversible impaired spermatogenesis, depending on the stage of differentiation affected by the chemical. Damage to spermatogonia causes irreversible impaired sperm production, because these stem cells are not replenished. A striking example of a pesticide acting as such is

the nematocide dibromochloropropane (DBCP) which caused azoospermia without recovery even after 7 years of follow-up (14). Damage to cells further in the differentiation process may lead to temporarily decreased fertility because of changes in the cell number, structure, motility, or viability of spermatozoa. These effects are transient because spermatogenesis is restored from stem cell populations after removal of the offending chemical (15). Pesticides can also affect Sertoli cells. Several authors found altered Sertoli cell function or changes in the morphological appearance of these cells after exposure to different pesticides in animal and in vitro models (16–22), but not all researchers found similar effects (23). The functional impairment, damage, or destruction of Sertoli cells is also very detrimental to spermatogenesis because these cells are essential for the proliferation and differentiation of all spermatogenic cells. Because Sertoli cells do not regenerate after puberty, extensive damage can lead to irreversible impairment of spermatogenesis. No directly damaging effects of pesticides on Leydig cells have been described in the literature. However, the function of Leydig cells can be impaired by pesticide exposure, the result being decreased testosterone concentrations in serum and testicular tissue (24–26). This occurrence, in turn, can lead to diminished Sertoli cell function and spermatogenesis. The mechanism through which these pesticides decrease testosterone synthesis is thought to be endocrine disruption, which may be the main mechanism underlying the effects of pesticides on reproduction.

Endocrine-disrupting chemical effects

An endocrine-disrupting chemical is defined as an exogenous agent that interferes with the synthesis, storage and release, transport, metabolism, binding, action, or elimination of natural blood-borne hormones that are responsible for the maintenance of homeostasis and the regulation of developmental processes in the body (27, 28). Human exposure to endocrine-disrupting chemicals occurs through multiple pathways with diet, drinking water, air, and skin as the most common routes of uptake of these chemicals into the body (29). In the next paragraphs, we review the mechanisms relevant for the pesticide-induced endocrine-disrupting effects on male reproduction observed in experimental animal studies (in vivo) and cell culture studies (in vitro).

Interference with hormone synthesis. Several pesticides are able to decrease steroidogenesis, for example, by inhibiting specific enzymatic steps in the biosynthesis pathway of these hormones (30). Examples of pesticides with this mechanism are dimethoate, roundup, and lindane, which all reduce steroidogenic acute regulatory (StAR) protein expression (26, 31, 32). This particular

protein mediates the transfer of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting and acutely regulating step in steroidogenesis. By blocking the expression of this protein, Leydig cells produce less testosterone in vitro. Another mechanism of action is the reduction of P450 cholesterol side-chain cleavage enzyme (P450_{sc}) activity by, for example, dimethoate, roundup, 2,2-bis(p-hydroxyphenyl)-1,1,1-trichloroethane, a metabolite of methoxychlor, and ketoconazole (26, 31, 33, 34). P450_{sc} is an enzyme that catalyzes the first reaction in the testosterone biosynthesis pathway: the conversion of cholesterol to pregnenolone. The imidazole fungicide ketoconazole has been shown to reduce multiple enzyme activities in testosterone biosynthesis, such as 17,20-desmolase, 17 α -hydroxylase, and 17 β -hydroxysteroid dehydrogenase, the result being a decrease in testosterone concentrations (35–39).

Interference with hormone storage and release. Interference with hormone storage and release is also mentioned in the definition of endocrine-disrupting chemicals as a mechanism of action. However, steroid hormones are not stored intracellularly within membranous secretory granules, but are readily synthesized after gonadotropin stimulation of the gonads. Therefore, the release of steroid hormones is primarily dependent on the activation of receptors on Leydig and Sertoli cells and the subsequent biosynthesis pathways (40).

Interference with hormone transport and clearance. For the most part, steroid hormones in the bloodstream do not float around freely, but are bound to carrier proteins, such as sex-steroid hormone-binding globulin (SHBG). Because only free hormones can be biologically active, increases or decreases in the concentration of SHBG have a major impact on the available free and active steroid hormone concentration in blood. Estrogens are known to increase SHBG concentrations in plasma, whereas androgens decrease these concentrations (27, 41). Substances that mimic these natural hormones can cause similar changes, but specific articles citing effects of pesticides on SHBG levels could not be found. However, reports have been published about the influence of pesticides on the clearance of steroid hormones that are catabolized by liver enzymes. Many pesticides induce the liver enzymes monooxygenase and UDP-glucuronosyltransferase (42) and result in increased clearance of the pesticide itself for detoxification purposes, but also an increased clearance of testosterone (43, 44). For example, DDT (dichlorodiphenyltrichloroethane) analogs are potent inducers of hepatic microsomal monooxygenase activity in vivo (45), which degrades endogenous androgens and results in suppressed androgen-receptor-mediated activity. These effects have also

been suggested for endosulfan and mirex (46). Similarly, treatment with lindane has been reported to increase the clearance of estrogens (47).

Interference with hormone receptor recognition and binding. Interference with hormone receptor recognition and binding is a mechanism of endocrine disruption that has been discussed a great deal in the literature. Some pesticides can interact with the steroid hormone family of nuclear estrogen receptors (ER) and androgen receptors (AR), which are both widely distributed in male reproductive tissues. These pesticides disrupt the natural ligand-receptor binding and thereby act as agonists through their chemical resemblance to the natural ligand or as antagonists, by blocking the receptor for endogenous hormones (48). Mostly, it involves endocrine-disrupting chemicals that possess estrogenic activity (the largest group) or anti-androgenic activity (49), but some endocrine-disrupting chemicals may have both estrogenic and anti-androgenic activity (49, 50). Examples of pesticides acting as estrogen agonists are endosulfan, toxaphene, dieldrin, o,p'-DDT, β -HCH, methoxychlor, chlordecone (Kepone), and dimethoate (46, 51–54). Endocrine-disrupting chemicals that may have anti-androgenic activity are, for example, vinclozolin, p,p'-DDE (p,p'-dichlorodiphenyldichloroethylene), and o,p'-DDT (55–58). Wong et al (59) also described androgenic (agonistic) activity of enanilid, a metabolite of vinclozolin, at high concentrations (59), but other literature on the androgen-agonistic activity of pesticides is scarce.

Interference with the thyroid function. Pesticides like chlorophenols, chlorophenoxy acids, organochlorines, and quinones have been shown to alter thyroid gland function and to reduce circulating thyroid hormone levels (60, 61). A reduction of thyroid hormone levels can compromise the catalytic activity of hepatic cytochrome P450 monooxygenases and result in an altered hepatic androgen metabolism (62).

Interference with the central nervous system. The central nervous system (CNS) is very important in the integration of hormonal and behavioral activity. Disturbances in these finely tuned mechanisms can severely impair normal adaptive behavior and reproduction. Since many pesticides are known to be neurotoxic, it is conceivable that these chemicals can disrupt the coordinating activity of the CNS by disrupting brain cell functions (27). Resulting neural disorders may lead to impotence, failure to achieve an erection, or difficulties with ejaculation, all compromising reproductive activity (63). Pesticides can also alter hypothalamic and pituitary function and thus also the secretion of GnRH, LH, and FSH in a more direct manner by modifying the feedback of endogenous

hormones. For example, it has been demonstrated that low-dose exposure to o,p'-DDT and methoxychlor can result in diminished hypothalamic and pituitary function in rodents (64, 65). Finally, it is postulated that any environmental compound mimicking or antagonizing steroid hormone action could presumably alter the glycosylation of LH and FSH and thereby reduce their biological activity (66).

Potential effects of occupational exposure to pesticides on semen quality and time to pregnancy

The studies described in the previous sections primarily involve laboratory animals (in vivo) or cell cultures (in vitro). Animal and in vitro studies are widely used and are often the first indicators of potential reproductive or developmental effects. However, the health risks for human populations may considerably differ because of differences in the exposure levels (67), reproductive issues, metabolism, body size, and lifespan, all of which makes it difficult to extrapolate from effects found in animals to effects that might be expected in humans (68). Recognizing that a pesticide has the potential to cause harm reveals only a hazard. The risk of this pesticide actually inducing a biological effect depends on its properties, but the effect will only occur when exposure reaches a particular level (67). Endocrine disruptors that accumulate in the body may eventually reach the higher threshold levels necessary for exertion of their biological effects. Another difficulty in human studies is that people can be exposed to endocrine disruptors in various ways, such as through iatrogenic exposure, endogenous estrogens, natural substances with estrogenic or androgenic activity (bioflavonoiden), and environmental endocrine disruptors like pesticides. In addition, it is feasible that interaction between endocrine disruptors plays a role when there is combined exposure (69). Therefore, the results of epidemiologic studies seldom pertain to specific pesticides, and firm conclusions about endocrine disrupting effects on the male reproductive system are lacking. Still, we have included an overview of the epidemiologic studies concerning known and suspected effects of occupational pesticide exposure on male reproduction. [See tables 1 and 2.]

Effects of pesticides on semen quality

The most well-known occupational testicular toxin is DBCP. The first reports about the adverse effects of DBCP on semen quality were published in 1977 when Whorton et al (70) observed oligozoospermia (sperm concentration $<20 \times 10^6/\text{ml}$) and azoospermia (no spermatozoa in ejaculate) in workers of a DBCP-producing factory in California in the United States. Workers with sperm counts beneath 1 million/ml had been exposed for

at least 3 years, whereas men with sperm counts above 40 million had been exposed for less than 3 months, an occurrence indicating a striking relation between exposure duration and sperm count (70). An additional study on these men indicated exposure-duration-dependent effects as well. Among a total of 107 exposed men, 13.1% showed azoospermia, 16.8% were severely oligozoospermic, and 15.8% were mildly oligozoospermic. Sperm motility and morphology were also affected after an average of 8 years of DBCP exposure (12). Similar results were obtained by Potashnik et al (11), who reported azoospermia after chronic DBCP exposure (2–10 years) in six DBCP factory workers, two of whom suffered from infertility and four of whom had decreased libido or impotence. Testicular biopsies revealed complete atrophy of the seminiferous epithelium and the Sertoli-cell-only syndrome. Also studying DBCP plant workers, Egnatz et al (71) found the duration of DBCP exposure to be inversely related to sperm count and testicular volume. In Costa Rica, approximately 1500 male banana plantation workers were medically diagnosed as infertile as a result of exposure to DBCP. This finding suggests a remarkably high incidence of 20–25% among banana workers in this zone (72). Infertility was mainly due to azoospermia, whereas the sperm of the oligozoospermic men had reduced motility and appeared to be immature.

Several follow-up studies were performed on DBCP as well. Eaton et al (14) reported permanent destruction of the germinal epithelium in DBCP-sterile men, as only two out of eight initially azoospermic workers produced spermatozoa after 5–8 years of nonexposure and none of the oligozoospermic workers progressed to better sperm production. Olsen et al (73) determined spermatogenic recovery after a maximal exposure duration of 18 months. Of 26 the azoospermic men, 73% showed signs of recovery of spermatogenesis after 11 years of nonexposure, of which two-thirds achieved normospermia. Normospermia was also observed in all of the previously oligozoospermic men. Correspondingly, Lantz et al (74) concluded that early elimination of DBCP exposure permits the recovery of spermatogenesis in a period of 18 to 21 months, whereas Potashnik & Porath (75) reported that sperm count recovery was apparent within 36 months to 45 months of nonexposure. Thereafter, no further improvements in sperm count were observed.

Another pesticide with clearly demonstrated male reproductive toxicity is the fumigant ethylene dibromide (EDB). As early as 1987, Ratcliffe et al (76) and Schrader et al (77) observed decreases in sperm count and in the proportions of viable and motile spermatozoa, as well as increases in semen pH and in the proportion of sperm with specific morphological abnormalities, among 46 men working in the papaya fumigation industry in

Table 1. Results of the literature review of occupational exposure to pesticides and semen quality. (DBCP = dibromochloropropane, EDB = ethylene dibromide; 2,4-D = 2,4-dichlorophenoxyacetic acid, IMPY = 2-isopropyl-4-methyl-6-hydroxypyrimidine, US = United States).

Study	Pesticides	Exposed population	Exposure	Reference population	Main results	Study design	Remarks
Whorton et al, 1977 (70)	DBCP	25 workers in DBCP production factory	Length of time working in factory	—	Increase in percentage of oligozoospermia, increase in percentage of azoospermia	Cross-sectional	Observed effects appeared to be related to duration of exposure to DBCP
Whorton et al, 1979 (12)	DBCP	107 workers in DBCP production factory	—	—	Increase in percentage of oligozoospermia and azoospermia, decrease in sperm motility and sperm morphology	Cross-sectional	Indications for exposure-duration-dependant effects
Potashnik et al, 1978 (11)	DBCP	6 workers in DBCP production factory with azoospermia	—	—	Increase in percentage of infertility and in percentage of decreased libido or impotence	Cross-sectional	Very small study
Egnatz et al, 1980 (71)	DBCP	232 workers in DBCP plants producing and handling	Level and likelihood of exposure based on job categories ^a	97 workers from same plant (no exposure to DBCP, but exposure to spermatoxic metals, fumes or radiation possible)	Increase in percentage of azoospermia, decrease in percentage of sperm count, no change in sperm morphology	Cross-sectional	Validity of exposure classification questionable
Thrupp, 1991 (72)	DBCP	Atlantic banana growing region of Costa Rica	Approximately 1500 male workers diagnosed as infertile as a result of exposure to DBCP	—	High incidence of 20–25% of infertility in this zone, increase in percentage of oligospermia and azoospermia, decrease in sperm motility	—	—
Eaton et al, 1986 (14)	DBCP	Follow-up of 44 workers who worked in DBCP production factory	—	—	2 of 8 originally azospermic workers produced sperm during the follow-up after 5–8 years of nonexposure; no increase in sperm production among men who had a low sperm count in 1977 after follow-up	Longitudinal (follow-up: 5–8 years)	Results suggest permanent destruction of germinal epithelium in most DBCP-sterile persons
Olsen et al, 1990 (73)	DBCP	75 workers in DBCP production factory	Duration <20 months; levels categorized by physician on basis of interviews and work history records [high (N=37), moderate (N=18) or low (N=20)]; no exposure during follow-up	49 workers in DBCP production factory categorized as unexposed by physician on basis of interviews and work history records	Decrease in sperm count and percentage of normospermic men; recovery of spermatogenesis in 19 out of 26 azospermic men during follow-up	Initial cross-sectional (follow-up: 6–11 years)	Exposure–response relationships for sperm count and sperm quality, recovery not associated with initial exposure level or duration of exposure, nonrecovery was job dependent
Lantz et al, 1981 (74)	DBCP	14 workers with oligospermia in DBCP production factory	Exposure to DBCP at most 30 months	—	Increase in sperm count after follow-up	Longitudinal (18–21 months)	No correlation between degree of impairment of semen quality with calculated hours of exposure to DBCP; level of DBCP exposure ?
Postashnik & Porath, 1995 (75)	DBCP	15 workers with DBCP-induced testicular dysfunction in DBCP production factory	Last contact with DBCP 17–22 years before last evaluation	—	Increase in sperm count recovery after follow-up	17 year re-evaluation	Sperm count recovery was more frequent among oligozoospermic men than azospermic men
Ratcliffe et al, 1987 (77); Schrader et al, 1987 (76); Schrader et al, 1988 (78)	EDB	46 fumigators at papaya plants	Duration 5 years, exposure level 88 ppb	43 paid volunteers with similar background from neighborhood sugar processing plant	Decrease in sperm count, nonsignificant decrease in sperm concentration, increase in percentage of oligospermic men, decrease in sperm motility, no change in sperm morphology, increase in percentage of abnormal shapes	Cross-sectional	Reference group consisted of first 43 volunteers, potential bias if rapid response correlated with known infertility, correction for several confounders like age and abstinence period

(continued)

Table 1. Continued.

Study	Pesticides	Exposed population	Exposure	Reference population	Main results	Study design	Remarks
Schrader et al, 1988 (78)	EDB	10 EDB-exposed forestry employees	Exposure time 6 weeks	6 unexposed forestry workers whose work duties did not allow contact with EDB fumigation operations	Sperm velocity decreased in all 10 exposed men and in only 2 unexposed men, semen volume decreased in 9 exposed men and in only 2 unexposed men	Longitudinal	Semen samples examined 1–2 weeks before exposure and during the last week of exposure to EDB; relevant time window questionable as spermatogenesis takes 74 days
Cannon et al, 1978 (79)	Kepone	133 workers in a Kepone-producing chemical plant	High doses of chlordecone	—	Increase in percentage of oligospermia, decrease in sperm morphology and sperm motility	Cross-sectional	Effects more often found in production workers than in nonproduction personnel; Kepone level was 2.53 ppm for workers with effects and 0.6 ppm for those without effects
Guzelian, 1982 (80)	Kepone	13 workers who had high concentrations of chlordecone (0.6–32.0 µg/g in blood)	—	—	Decrease in sperm motility, increase in number of motile sperm as blood level of chlordecone decreased	Cross-sectional	Results indicate that the effects of chlordecone not permanent
Whorton et al, 1979 (81)	Carbaryl	47 workers in a carbaryl production plant	Minimum exposure 1 year; in absence of detailed industrial hygiene exposure data, a subjective exposure classification was developed	Historical control group of unexposed men from three other workplace studies	No effects on semen parameters	Cross-sectional	Comparability of control group ?
Wyrobek et al, 1981 (82)	Carbaryl	50 workers in a carbaryl production plant	Duration 1–18 years; operation area 4.9 mg/m ³ ; distribution area 0.35 mg/m ³ ; exposure groups for low dose: supervisors, foremen, vacation; exposure groups for high dose: full-time baggers and operators; number of work years	34 newly hired workers in a carbaryl production plant; these workers gave semen samples during their preemployment medical examinations before assignment to the chemical plant	Nonsignificant increase in percentage of oligospermic men, no effect on sperm count, decrease in sperm morphology	Cross-sectional	Correction for age, smoking habits and medical problems; no dose-response relation with sperm morphology; negative correlation between number of years working in carbaryl area and percentage of abnormal sperm; misclassification of exposure ?
Pa-dung-tod et al, 2000 (83)	3 organophosphate pesticides (methyl parathion, ethyl parathion, me-thamido-phos)	20 workers in a pesticide factory	Low exposure in production line monitoring, high exposure in packing	23 unexposed workers from a nearby textile factory	Decrease in sperm concentration, sperm count, sperm motility, nonsignificant decrease in sperm morphology	Cross-sectional	Weak evidence of a dose-response relationship between urinary p-nitrophenol (internal dose) and sperm parameters; correction for age, abstinence period, and current smoking; exposure assessment by biological monitoring
Ler-da & Rizzi, 1991 (84)	2,4-D (herbicide)	32 farm sprayers	Estimated by measuring concentrations of 2,4-D in urine; mean level of 2,4-D: 9.02 mg/l	25 controls	Increase in percentage of asthenospermia, percentage of necrospermia, and percentage of teratospermia and decrease in sperm motility—after short recovery period, asthenospermia and necrospermia disappeared and motility increased	Cross-sectional (farmers: longitudinal)	Correction for confounders ?; comparability of control group ?
Tomen-son et al, 1999 (85)	Molinate (thiocarbamate herbicide)	272 formulation and production workers at three US plants	Mean exposures: 12.7–210.9 µg/m ³ ; exposure for each period calculated by multiplication of geometric mean exposure to molinate vapor by number of hours of molinate exposure	—	No effects on semen parameters	Longitudinal	Correction for fever, smoking history, alcohol consumption, and use of sauna or hot baths—solid analysis; solid exposure assessment

(continued)

Table 1. Continued.

Study	Pesticides	Exposed population	Exposure	Reference population	Main results	Study design	Remarks
Abell et al, 2000 (86)	Mixture of pesticides	122 greenhouse workers from 30 ornamental flower greenhouses	Ratio between area treated with pesticides during 3 months prior to semen sample collection and total greenhouse area; classification according to job task and work practice; workers ranked to 3 groups according to transfer of pesticide residues from cultures to hands	—	Intensity of pesticide: exposure unrelated to semen characteristics; high re-entry exposure: decrease in sperm concentration and sperm morphology; sperm concentration, viability, and motility inversely related to total duration of work in greenhouses	Cross-sectional	Correction for age, urogenital disease, fever, and spillage; no control group; misclassification of exposure ?; sperm morphology improved with long-term exposure to pesticides
Larsen et al, 1999 (87)	Mixture of pesticides	171 traditional farmers	95% sprayed >3 months before semen sample	85 organic farmers (exposure: 67% used pesticides in the past)	No effect on sperm concentration and sperm motility, decrease in sperm morphology, no effect on proportion of dead spermatozoa	Cross-sectional	Semen samples collected in spring before start of spraying season; correction for several confounders
Larsen et al, 1998 (88)	Mixture of pesticides	161 traditional farmers spraying pesticides	—	87 organic farmers not spraying pesticides	Sperm morphology increased in spraying farmers and decreased in nonspraying farmers; no associations between sperm parameters and exposure variables; decrease in sperm morphology (exposure to herbicides >12 hours)	Longitudinal	Semen sample before spraying season and 12 to 16 weeks after first spraying day; lack of sufficient exposure contrast ?; misclassification of exposure; correction for several confounders
Kamijima et al, 2004 (89)	Insecticides, mainly organophosphorus and pyrethroid	18 male indoor pesticide sprayers who worked for 9 companies	—	18 age-matched students or medical sectors	No effect on sperm count, sperm concentration and sperm vitality; decrease in sperm motility (in summer)	Longitudinal	Semen samples collected in summer (busiest season for pesticide spraying) and in winter (off-season); comparability of control group ?; no exposure assessment; only corrected for abstinence period
Swan et al, 2003 (90)	Mixture of pesticides	50 cases with low sperm concentration, percentage of normal morphology, and percentage of motility	Urine samples collected at same time as semen samples were provided to measure 8 currently use pesticides	36 controls with normal semen parameters	Higher concentrations of pesticide metabolite levels among cases than controls; high levels of alachlor, IMPY, and atrazine associated with case status	Case-control	Cause-effect relation ?; only 3 men reported occupational pesticide exposure; correction for confounders ?
Tielemans et al, 1999 (91)	Various occupational exposures, including pesticides	Male partners of couples having their first consultation at two infertility clinics (N=899), divided into cases according to 3 different cutoff points: (A) concentration <20 × 10 ⁶ /ml or motility <50% or morphology <14%, (B) concentration <5 × 10 ⁶ /ml or motility <10% or morphology <5%, and (C) azoospermia	Occupational exposure assessed using job-specific questionnaires, a job exposure matrix, and measurements of metabolites in urine	Concentration of ≥20 × 10 ⁶ /ml and ≥50% motility and ≥14% morphology	No associations between exposure to pesticides and reduced semen quality	Case-control	Only 12 men exposed to pesticides; correction for several confounders; selection bias ?; misclassification of exposure by job title and questionnaire data

(continued)

Table 1. Continued.

Study	Pesticides	Exposed population	Exposure	Reference population	Main results	Study design	Remarks
Strohmer et al, 1993 (92)	Agricultural work	Spouses of 103 females opting for artificial insemination with donor sperm because of proven poor sperm quality or azoospermia (cases)		Spouses of 103 females requiring in vitro fertilization because of tubal factors or other female causes of subfertility (controls)	Cases: 11 of 103 with agricultural work; controls: 1 of 103 with agricultural work	Case-control	Selection bias and confounding ?; misclassification of exposure by job title and questionnaire data
Henderson et al, 1986 (93)	Various chemicals including pesticides	1695 men with abnormal semen characteristics or suspicion of male fertility disorders attended a reproductive medicine clinic		—	Agricultural workers: decrease in sperm concentration, no effect on sperm morphology and sperm motility	Cross-sectional	Selection bias and confounding ?; misclassification of exposure by job title and questionnaire data
Oliva et al, 2001 (94)	Various chemicals, including pesticides	177 men consulting infertility clinics because of male infertility	40 men exposed to pesticides in this population, with median exposure time of 7 years	—	Exposure to pesticides significantly associated with sperm threshold values below the limit for male fertility; associations higher in frequently exposed men than in occasionally exposed men	Cross-sectional	Selection bias and confounding ?; misclassification of exposure by questionnaire data
Wong et al, 2003 (95)	Various chemicals, including pesticides	73 men consulting a fertility clinic with sperm counts of 5–20 million/ml from couples who tried to conceive >1 year (subfertile group)		92 men recruited from 9 midwifery practices with a sperm count of >20 million/ml whose partners conceived within 1 year (fertile group)	Pesticide exposure was associated with oligospermia	Case-control	Only 12 men exposed to pesticides—misclassification of exposure by questionnaire data; controls not comparable to cases regarding catchment area (local versus regional)

^a Study performed >20 months after termination of production.

Table 2. Results of the literature review on occupational exposure to pesticides and time to pregnancy. (95% CI = 95% confidence interval, DDT = dichlorodiphenyltrichloroethane, HCB = exachlorobenzene, IVF = in vitro fertilization)

Study	Pesticides	Exposed population	Exposure	Reference population	Main results	Study design	Remarks
De Cock et al, 1994 (10)	Mixture of pesticides including fungicides (eg, captan), herbicides (eg, simazin) and insecticides	19 male fruit growers	Application of pesticides classified as high exposure on basis of low spraying velocity; duration seasonal	24 male fruit growers (exposure: application of pesticides classified as low exposure on basis of high spraying velocity)	Fecundability ratio 0.47 (95% CI 0.28–0.77)	Cross-sectional	Time-to-pregnancy history; extensive exposure assessment—effect modification of spraying; season in high exposure; potential exposure of wives ?
Thonneau et al, 1999 (97)	Mixture of pesticides (vineyard workers: fungicides; farmers: herbicides; greenhouse workers: fungicides, insecticides and growth regulators)	France: 142 rural workers; Denmark: 326 conventional farmers, 121 greenhouse workers	Exposed to pesticides during calendar year before birth of youngest child in France; exposed to pesticides during calendar year before birth of youngest child in Denmark	220 French rural workers and 123 Danish conventional and organic farmers not exposed to pesticides during calendar year before birth of youngest child	France: fecundability ratio for rural workers 1.17 (95% CI 0.89–1.55); Denmark: fecundability ratio for conventional farmers 1.09 (95% CI 0.82–1.43) and for greenhouse workers 0.83 (95% CI 0.69–1.18)	Retrospective	Youngest child potential for bias related to time to pregnancy; no exposure assessment; misclassification of exposure; correction for confounders

(continued)

Table 2. Continued.

Study	Pesticides	Exposed population	Exposure	Reference population	Main results	Study design	Remarks
Larsen et al, 1998 (98)	Mixture of pesticides (farmers: herbicides; greenhouse workers: fungicides, insecticides and growth regulators)	450 traditional farmers spraying pesticides	Exposed to pesticides during calendar year before birth of youngest child	72 traditional farmers not spraying pesticides themselves and 94 organic farmers not exposed to pesticides during calendar year before birth of youngest child	Fecundability ratio 1.18 (95% CI 0.83–1.66); fecundability ratio 1.03 (95% CI 0.75–1.40)	Retro-spective	Youngest child: potential for bias related to time to pregnancy; no exposure assessment; correction for confounders
Curtis et al, 1999 (99)	Mixture of pesticides including herbicides (eg, glyphosate and atrazine), insecticides (eg, carbaryl), and fungicides (eg, captan)	1048 farm occupants → 2012 pregnancies	4 different exposure groups based on reported pesticide use on farm combined with pesticide activities reported by husband or wife around the time they started trying to conceive	1048 farm occupants → 2012 pregnancies (exposure: no pesticide use on farm and no pesticide activities reported by husband or wife)	No strong or consistent pattern of associations between time to pregnancy and pesticide exposure among different groups	Retro-spective cohort	All pregnancies of participants: potential for dependent outcomes; exposure assessment; misclassification of exposure ?
Rupa et al, 1991 (100)	Mixture of pesticides including DDT, HCB, and organophosphorus pesticides	1016 cotton field workers	Mixing and backpack spraying of pesticides without protective equipment; duration seasonal	1020 males with similar socioeconomic status (no exposure to pesticides)	Increase in percentage of infertile males	Cross-sectional	Effect modification by smoking; selection bias and confounding ?
Heacock et al, 1998 (101)	Chlorophenolate fungicides (mainly sodium tetra- and pentachlorophenates)	23829 sawmill workers of 11 large lumber mills	Exposed to chlorophenolate fungicides	2658 unexposed sawmill workers	Mantel Haenzel rate ratio 0.89 (95% CI 0.84–0.93)	Retro-spective	Exposure misclassification ?; no correction for confounders
Petrelli & Figa-	Mixture of pesticides	127 greenhouse workers	Low exposure: 1 to 100 hours of pesticide application per year; high exposure: >100 hours of pesticide application per year; duration: throughout the year	173 clerical workers in same geographic area (no exposure to greenhouse work)	Odds ratio: low exposure 1.6 (95% CI 0.8–3.1), high exposure 2.4 (95% CI 1.2–5.1)	Retro-spective	First pregnancy !; exposure assessment ?; misclassification of exposure
Sallmén et al, 2003 (103)	Mixture of pesticides including herbicides and insecticides	210 greenhouse and garden workers	No exposure: worker did not report any pesticide application or handling of treated plants; low exposure (N=65): worker sprayed pesticides once a month or worked with treated plants less than once a week; moderate exposure (N=28): worker applied pesticides 2–3 times a month or handled treated plants 1–2 days a week; high exposure (N=50): worker applied pesticides at least once a week or worked with treated plants 3 days a week	312 unexposed workers (no work in greenhouses or gardens)	Fecundability density ratio: work in greenhouses 0.86 (95% CI 0.62–1.19), low exposure 0.77 (95% CI 0.46–1.29), moderate exposure 0.92 (95% CI 0.45–1.88), high exposure 0.67 (95% CI 0.33–1.35); exposure: pyrethroids 0.40 (95% CI 0.19–0.85), organophosphates 0.70 (95% CI 0.42–1.17), carbamates 0.55 (95% CI 0.27–1.11)	Historical prospective	Exposure assessment !; correction for confounders
Tielemans et al, 1999 (104)	Mixture of pesticides	836 couples who sought IVF treatment between 1991 and 1998	20 men potentially exposed to pesticides filled in a job-specific questionnaire and details were subsequently collected by telephone interview; moderately exposed (N=9); highly exposed (N=7)		Decrease in fertilization rate; odds ratio: potential exposure 0.54 (95% CI 0.29–0.99), confirmed exposure 0.38 (95% CI 0.19–0.78), moderately exposed 0.52 (95% CI 0.22–1.24), highly exposed 0.22 (95% CI 0.06–0.80)	Case-control	Only couples who sought IVF treatment: selection bias ?

Hawaii when these men were compared with 43 unexposed men from a nearby sugar refinery. Moreover, sperm viability and motility were poorer among the men with longer EDB exposure. Schrader et al (78) also conducted a longitudinal study on the effects of EDB on semen quality; in their study a reduction in sperm

motility and semen volume was found in all of the 10 exposed men and in 2 of the 6 unexposed men.

The third pesticide known to cause alterations in semen quality is the chlorinated hydrocarbon insecticide chlordecone (Kepone). In a study conducted in 1974, Cannon et al (79) characterized an outbreak of a new

disease in 57% of 133 persons working in a Kepone-producing chemical plant with virtually uncontrolled exposure to high doses of chlordecone. Semen samples revealed oligozoospermia with predominating abnormal and nonmotile spermatozoa. Four years later, Guzelian (80) reported that treating patients with cholestyramine, which binds to chlordecone, recovers the number of motile sperm, this finding indicating that the effects can be reversed upon removal of the pesticide from the body.

Other, less investigated pesticides thought to interfere with human spermatogenesis are carbaryl, the organophosphate pesticides methyl parathion, ethyl parathion and methamidophos, 2,4-dichlorophenoxyacetic acid (2,4-D), and the thiocarbamate molinate. Human studies on the effects of carbaryl on spermatogenesis yielded conflicting results. Whorton et al (81) examined 47 workers employed for a minimum of 1 year in a carbaryl production plant and found no adverse effects of exposure on any semen parameter. However, Wyrobek et al (82) reported increased proportions of abnormally shaped sperm in exposed versus unexposed workers (52.0% versus 41.9%) in basically the same cohort. Padungtod et al (83) investigated the effects of the three organophosphate pesticides on spermatogenesis among 20 exposed workers employed at a Chinese pesticide manufacturing plant in comparison with 23 unexposed workers from a nearby textile factory. The largest effect was observed on sperm concentration ($28.5 \times 10^6/\text{ml}$ for the exposed and $49.4 \times 10^6/\text{ml}$ for the unexposed). Sperm count, percentage of motile spermatozoa, and percentage of sperm with normal morphology were modestly lower among the exposed workers. The adverse effects of the herbicide 2,4-D were evaluated by Lerda & Rizzi (84). Asthenospermia, necrospermia, and teratospermia were all more frequent among exposed farm sprayers than among unexposed controls, while motility was affected as well. Interestingly, asthenospermia and necrospermia disappeared and motility increased after a short recovery period, but teratospermia remained. Tomenson et al (85) investigated the adverse effects of the thiocarbamate herbicide molinate among 272 formulation and production workers at three plants in the United States and concluded that sperm parameters were not related to molinate exposure.

Other investigators studied occupational groups working with mixtures of pesticides, rather than focusing on specific pesticides. Abell et al (86) analyzed semen samples from 122 greenhouse workers with exposure to several pesticides, including primary exposure during mixing and spraying or secondary (re-entry) exposure during the handling of cultures. Although no relation was found between the semen characteristics and the overall exposure intensity, the workers with the highest estimated re-entry exposure had lower sperm concentrations and proportions of normal spermatozoa

than the low-level exposure group. Furthermore, inverse associations were observed between the total duration of work in greenhouses and sperm concentration, viability, and motility. Surprisingly, the authors also observed that sperm morphology improved with long-term exposure to pesticides.

A study conducted by the ASCLEPIOS study group compared semen quality in 85 organic farmers with that of a group of 171 traditional farmers before the spraying season (87). The results did not indicate differences in sperm concentration and sperm motility, but traditional farmers seemed to have a lower proportion of normal sperm heads than organic farmers (39.5% versus 42.3%) according to the WHO scoring system and a lower proportion of normal spermatozoa (2.5% versus 3.4%) according to the strict criteria. They also investigated short-term within-person changes in semen quality during the spraying season in their population of traditional and organic farmers (88). During this period, both groups showed a comparable decline in sperm concentration, which was attributed to seasonal variability. Remarkably, the spraying farmers had an increase in normal sperm morphology over the spraying period, while a decrease was found among the nonspraying farmers. Traditional farmers with exposure durations exceeding 12 hours had a lower proportion of normal sperm and curvilinear velocity of spermatozoa than the farmers exposed for less than 12 hours during the time period between the two semen samples (12–16 weeks).

In Japan, Kamijima et al (89) compared semen samples of 18 male indoor insecticide sprayers with 18 unexposed age-matched students or physicians. Sperm counts and vitality were comparable between the groups, but detailed sperm motility analyses in summer revealed that the percentages of slow progressive and nonprogressive motile sperm were twice as high in sprayers, while the percentages of rapidly progressive sperm tended to be lower. A different approach was taken by Swan et al (90), who addressed the hypothesis that the reduced sperm concentration and motility observed in fertile men in an agricultural area in the United States could be explained by pesticide use. They compared 50 cases with a low sperm concentration, low percentages of normal sperm morphology, and low percentages of motile sperm to 36 controls whose semen parameters were within normal limits. Strong positive associations were found between case status and high urine levels of alachlor, 2-isopropoxy-4-methyl-pyrimidinol, and atrazine. High levels of metolachlor metabolites were weakly associated with case status as well, while high levels of acetochlor were associated with control status. In addition, case status was linked with exposure to multiple pesticides (90).

Other researchers focused on subfertile men visiting fertility clinics to study associations between pesticide

exposure and semen characteristics. Using job-specific questionnaires and telephone interviews, Tielemans et al (91) collected data about pesticide exposure from male partners of infertile couples in the Netherlands. Of the 899 participants, only 12 men were exposed to pesticides and no associations were found with reduced semen quality. Strohmer et al (92) compared the occupations of spouses with proved poor sperm quality among 103 females opting for artificial insemination with donor sperm and spouses of 103 females requiring in vitro fertilization (IVF) due to female causes of subfertility. The case group included 11 agricultural workers, compared with 1 among the controls. The authors concluded that the conspicuously high prevalence of agricultural workers among the cases could be explained by increased exposure to chemical sprays. Another study reporting that farmers exhibited the lowest sperm counts among patients of an infertility clinic confirms these findings (93). In the Litoral Sur region in Argentina, an area with intensive agricultural and industrial activity, Oliva et al (94) investigated a population of 177 men consulting infertility clinics because of male infertility; 40 of these men were exposed to pesticides with a median exposure time of 7 years. Among the men with primary subfertility, pesticide exposure was associated with a sperm concentration of <1 million/ml, $<50\%$ motile sperm, and $<30\%$ morphologically normal spermatozoa. All of the associations were stronger for the frequently exposed men than for the occasionally exposed men (94). A study (95) conducted among 73 men consulting an infertility clinic because of male factor subfertility concluded that pesticide exposure was associated with oligozoospermia. However, the number of exposed men was only 12, and the control group was not comparable with the cases.

To summarize, several studies have been conducted to evaluate the effects of pesticides on semen quality. For three pesticides (DBCP, EDB, and Kepone) major effects were observed for semen parameters, which could easily be detected in small studies because the effects of the pesticides were so catastrophic. However, uncovering more subtle effects of other pesticides requires different study designs and larger numbers of men, because of the large inter- and intraindividual variation in semen quality. Most of such studies done so far did not include proper control groups or detailed measurements of pesticide exposure either, so severe misclassification could have influenced the results. Therefore, the absence of evidence for effects of occupational pesticide exposure on semen quality could be misleading (96).

Effects of pesticides on time to pregnancy

The effects of most pesticides on semen parameters reviewed in the previous section cannot be directly associated with fertility or subfertility among the men

involved. However, an indication of the effect on male fertility can be obtained by assessing time to pregnancy, which is a measure of couple fecundability and includes both male and female effects. Unfortunately, most time-to-pregnancy studies do not include actual measurements of pesticide exposure, and the results are difficult to compare because of the heterogeneity of the exposure and study design.

De Cock et al (10) explored the association between male exposure to pesticides and time to pregnancy among 43 fruit growers in the Netherlands, providing information on 91 pregnancies during 1978–1990. High exposure to pesticides was found to be associated with a longer time to pregnancy, especially when the farmers tried to conceive during the spraying season from March to November. This seasonal effect supports a relation between the intensity of pesticide exposure and fecundability and suggests that the decrease in fecundability is reversible. However, the authors could not rule out that part of the observed effects may have been mediated by exposure of the wives to pesticides. A similar study was conducted by Thonneau et al (97) in France and Denmark, in which exposure was described as the use of pesticides during the calendar year before the birth of the youngest child among 362 French vineyard workers, 449 Danish conventional farmers, and 121 Danish greenhouse workers, who were primarily exposed to fungicides and insecticides. No differences in time to pregnancy were found between the pesticide exposed and unexposed workers in any of the populations. However, the exposure window was not optimal, as the exposure was assessed for the calendar year before the birth of the child and this period may not have included the attempt time for less fertile couples. In Denmark, comparisons were also made between (i) traditional farmers spraying pesticides (N=450), (ii) traditional farmers not spraying pesticides themselves (N=72), and (iii) organic farmers (N=94) (98). Again, the authors concluded that there was no overall effect of pesticides on male fecundability.

Among 1048 farm occupants in Canada who had 2012 planned pregnancies, Curtis et al (99) examined the association between time to pregnancy and pesticide use in the 2 months prior to the attempt to conceive. Overall, the authors found no strong or consistent pattern of associations, but, among the male farm workers engaged in pesticide activities, four pesticides were associated with a 12–15% decrease in fecundability [4-(2,4-dichlorophenoxy)butyric acid, cyanazine, fungicides, and captan]. Furthermore, three pesticides were associated with a 17–20% increase in fecundability (dicamba, glyphosate, and “other pesticides”), possibly due to uncontrolled confounding or the hormonal actions of these pesticides. In contrast, a study on male fertility among 1016 highly exposed cotton field workers in India and 1020 unexposed couples revealed that,

in the exposed group, the number of fertile men was significantly reduced when compared with the number in the control group (100). Heacock et al (101) assessed male fertility among 23 829 sawmill workers exposed to chlorophenolate fungicides in 11 large lumber mills in British Columbia in a comparison with 2658 unexposed sawmill workers. No inverse relationship was observed between cumulative exposure to chlorophenolate fungicides and fertility. With increasing cumulative exposure, fertility even seemed to increase; the authors attributed the increase to a type of healthy worker effect.

In Italy, Petrelli & Figà-Talamanca (102) studied time to pregnancy in relation to first pregnancy among 127 male greenhouse workers and 173 unexposed workers because greenhouse workers experience continuous pesticide exposure throughout the year, in contrast to agricultural workers. The average number of children was 1.8 for the exposed workers and 1.5 for the unexposed, and the mean time to pregnancy was 5.4 months and 3.9 months, respectively. The time to pregnancy for the greenhouse workers not using protective equipment was slightly longer, and workers with more than 100 applications per year had an increased risk of conception delay. In Finland, Sallmén et al (103) conducted a historical prospective study on the effects of pesticide exposure on time to pregnancy. In this study, 210 greenhouse and garden workers were compared with 312 men not exposed to pesticides. The results suggested a decrease in fecundability among the greenhouse and garden workers, especially for exposed greenhouse workers who wore inefficient protection. The exposed men who efficiently used personal protective equipment were as fertile as the unexposed greenhouse workers. Subgroup analyses yielded an association between fecundability and exposure to pyrethroids and suggestive associations between fecundability and the use of organophosphates and carbamates. Tielemans et al (104) focused on couples who sought in vitro fertilization to study the effect of paternal pesticide exposure on fertilizing ability in the Netherlands. Fertilization rates were decreased for couples with male partners occupationally exposed to pesticides. This finding suggests that paternal pesticide exposure decreases sperm fertilizing ability in vitro, although it was not possible to draw conclusions as to which chemical may have been responsible for this effect.

Discussion

In this review, we have described the mechanisms through which pesticides affect male fertility. Pesticides comprise a large number of distinct substances with dissimilar structures and diverse toxicity which act through

different mechanisms. Therefore, several of the aforementioned mechanisms are probably involved in the pathophysiological pathways explaining the role of pesticide exposure in effects on sperm quality and male fertility. A disadvantage of the studies on the mechanisms described is that they were primarily laboratory animal and cell culture studies. These types of studies often provide the first indications of potential reproductive effects of a chemical, but it is difficult to extrapolate the effects found in laboratory animals to effects that might be expected in men. Therefore, we also provided an overview of epidemiologic studies investigating occupational exposure to pesticides in relation to sperm quality and prolonged time to pregnancy. In these studies, clear effects on male fertility were only demonstrated for some pesticides (eg, DBCP and ethylene dibromide). Because the effects of these pesticides were so catastrophic, they could relatively easily be detected in small studies or through case reports. However, modern pesticides are supposed to be less toxic to humans so that more subtle effects of these pesticides on semen parameters will not come forth so easily. In this context, it is important to note that epidemiologic studies focusing on male fertility are confronted with large inter- and intraindividual variation in semen quality (105), potentially obscuring relations between exposure and effect. In addition, exposure assessment of pesticides is extremely complicated, as a large number of chemicals is involved. This complicated nature may have resulted in severe misclassification when crude methods of exposure assessment were used. As a matter of fact, the most important criticism of most studies in this overview is that occupational exposure to pesticides was only roughly characterized by dichotomies (eg, exposed or unexposed to pesticides, application of pesticides or not, or organic farmer versus traditional farmer). Therefore, it is no surprise that the results from recent studies are inconsistent with respect to the relation between pesticide exposure and semen quality. Moreover, most studies that found an association used a cross-sectional design, which has several severe limitations, such as selection bias due to differential participation and lack of information about the time dimension of the cause–effect relation (106).

In addition to potentially inadequate exposure assessment, studies on time to pregnancy have their own advantages and disadvantages. Time to pregnancy provides an estimate of the per cycle probability of conceiving a clinically detectable pregnancy. An increase in time to pregnancy can indicate reproductive loss at any of several different stages, including gametogenesis, transport of gametes in both male and female reproductive tracts, fertilization, migration of the zygote to uterus, implantation, and early survival of the concepts, which makes time to pregnancy a useful tool in identifying toxic effects on reproduction. In most of the time-to-pregnancy

studies reviewed, however, only men or women who had been pregnant were included; this procedure leads to the exclusion of sterile couples and an underestimation of the proportion of subfertile couples with long time to pregnancy. Another limitation of these time-to-pregnancy studies is that the following seven subtle, but potentially important sources of bias may have occurred and distorted the inferences made, especially in studies investigating the most recent pregnancy or all pregnancies of a couple: behavior modification bias, time trend bias, planning bias, wantedness bias, pregnancy recognition bias, medical intervention bias, and the reproductively unhealthy worker effect (107–110). Therefore, no uniform conclusions can be drawn about the effects of pesticides on time to pregnancy either.

In future research on time to pregnancy, we recommend that first pregnancies only be studied or that specific data on family history be collected. In addition, investigators in this field should put special emphasis on the adequate exposure assessment of pesticides. Biological monitoring could be one solution, but only when exposure to a limited number of different pesticides is involved. Among workers who are exposed to several different pesticides, using a semi-quantitative exposure assessment method, such as the Dermal Exposure Assessment Method (DREAM) (111), may be more feasible. Within such a method, various specific pesticides with similar mechanisms or modes of action can be selected. Furthermore, different time windows can be considered relevant for the total duration of spermatogenesis and varying waiting times to pregnancy to better elucidate the role of occupational pesticide exposure in male fertility.

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Appendix 1

Etiological factors related to male infertility and subfertility

Pretesticular factors

Endocrine
Hypogonadotropic hypogonadism

Coital disorders
Erectile dysfunction
Psychosexual
Endocrine/neural/vascular
Ejaculatory failure
Psychosexual
Postgenitourinary surgery
Neural
Drug-related

Testicular factors

Absence of testicular tissue or cells
Anorchism
Bilateral castration
Sertoli-cell-only syndrome

Genetic
Klinefelter's syndrome
Y chromosome deletions
Autosomal rearrangements
Immotile cilia syndrome
Partial androgen insensitivity

Congenital
Cryptorchidism

Infective
Orchitis

Antispermato-genic agents
Heat
Chemotherapy
Drugs/alcohol/tobacco
Irradiation
Pesticides

Vascular
Torsion
Varicocele

Immunological

Testicular cancer

Idiopathic

Post-testicular factors

Obstructive
Epididymal
Congenital
Infective
Vasal
Genetic: cystic fibrosis
Acquired: vasectomy

Epididymal hostility
Epididymal asthenozoospermia

Accessory gland infection

Immunological
Idiopathic
Postvasectomy

Disturbances in spermoocyte fusion

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