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Effects of environmental and occupational pesticide exposure on human sperm: a systematic review

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Relatively recent discoveries of the hormone disrupting properties of some pesticides have raised interest in how contemporary pesticide exposures, which primarily take the form of low level environmental or occupational exposures, impact spermatogenesis. The objective of the present review was to summarize results to date of studies examining pesticide effects on human sperm. Outcomes evaluated included sperm parameters, DNA damage and numerical chromosome aberrations (aneuploidy (disomy, nullisomy) or diploidy). Studies investigating sperm in men environmentally and/or occupationally exposed to any types of pesticides were included in the review. The targeted literature search over the last 15 years showed a range of pesticide classes have been investigated including pyrethroids, organophosphates, phenoxyacetic acids, carbamates, organochlorines and pesticide mixtures. None of the studies involved acute exposure events such as chemical accidents. There were 20 studies evaluating semen quality, of which 13 studies reported an association between exposure and semen quality; 6 studies evaluating DNA damage, of which 3 reported an association with exposure; and 6 studies assessing sperm aneuploidy or diploidy, of which 4 reported an association with exposure. Studies varied widely in methods, exposures and outcomes. Although suggestive for semen parameters, the epidemiologic evidence accumulated thus far remains equivocal as to the spermatotoxic and aneugenic potential of pesticides given the small number of published studies. This question warrants more investigation and suggestions for future studies are outlined.

Keywords: semen quality; chromosomes; DNA damage; environmental health; occupational health

Introduction

The health effects of pesticide exposures on male reproduction are a topic of considerable concern in environmental, occupational and reproductive epidemiology. In recent years, scientists have become more aware that human-made chemicals may disrupt reproductive function in both wildlife and humans (Colborn *et al*., 1993; Golden *et al*., 1999; Moline *et al*., 2000). Pesticides, as human-made chemicals designed to kill living target organisms, are biologically active. An early insight into how pesticides can act as reproductive toxicants at the population level came from case reports in the 1970s of sterility among men working with the nematicide dibromochloropropane (Teitelbaum, 1999). Relatively recent discoveries of the hormone disrupting properties of some pesticides at low exposures (Kelce *et al*., 1994; Anway *et al*., 2005) have raised interest in how low-dose acute or chronic pesticide exposures—the types of exposures that can currently occur both occupationally and environmentally—impact human spermatogenesis.

Although both animal toxicology and human epidemiologic studies have shown that pesticides may operate through hormonal or genotoxic pathways to affect spermatogenesis (Toppari *et al*., 1996), a limited number of epidemiologic studies have been published. The objective of the present review was to evaluate population based studies over the past 15 years to determine the weight of evidence for associations between occupational and environmental pesticide exposures and different sperm indicators including semen quality, DNA damage and numerical chromosome aberrations.

Scope of pesticide use

Pesticides are a broad group of biologically active chemicals used for pest management. It is estimated that there are 1844 pesticide compounds currently in commercial use in the USA. Pesticides can affect human health, and short-term acute exposure effects have been well documented. Small amounts of some of these chemicals cause death (Brandt *et al*., 2001); disrupt hormones and reduce the ability to successfully reproduce (Bonde, 2002; Claman, 2004; Sharpe and Irvine, 2004); and have been associated with specific cancers (Fleming *et al*., 2003; Alavanja *et al*., 2004). The World Health Organization (Dinham and Malik, 2003) estimates that 20 000 women, men and children die of accidental pesticide poisonings each year; three million are non-fatally poisoned and nearly three-fourths of a million new people each year experience chronic effects from exposure.

Semen quality indicators

Reproductive toxicology studies have focused on measuring semen parameters because chemical interference with sperm concentration, sperm motility and sperm morphology may impede fertilizing capability. Standard methods for collecting, handling, preparing and analyzing sperm samples manually have been established by the World Health Organization (1999) and are widely used. Age and abstinence time have been found to be related to semen volume, sperm concentration and sperm motility (Magnus *et al*., 1991; Eskenazi *et al*., 2003).

Markers of DNA damage in sperm

Although the analysis of semen parameters may provide some indication of the function of the testis and sperm, it does not provide information on the condition of the male genome contained in sperm heads (Morris *et al*., 2002). The integrity of sperm DNA is central to the transmission of genetic information during reproduction and chromatin abnormalities or DNA damage can result in paternal fertility problems (Evenson *et al*., 2002; Agarwal and Said, 2003). Sperm chromatin structure assay (SCSA) is considered highly stable and robust, having the least interassay variation compared with other methods for measuring DNA integrity (Evenson *et al*., 2002; Perreault *et al*., 2003). Endogenous nicks in the DNA of ejaculated spermatozoa, from double and single DNA strand breaks identified by terminal deoxynucleotidyl transferase-mediated dUDP nick-end labeling (TUNEL) are also indicative of DNA damage and this assay has been highly correlated with reproductive outcomes (Henkel *et al*., 2004). Similar relationships to fertilizing ability have been demonstrated using single cell gel electrophoresis or the neutral comet assay, as an indicator of DNA damage (Morris *et al*., 2002). Both age and abstinence time are established determinants of sperm chromatin abnormalities (Evenson *et al*., 2002).

Numerical chromosome aberrations in sperm

Possible reproductive toxicants such as pesticides may affect the normal disjunction of chromosomes during meiosis, therefore altering the number of chromosomes in sperm nuclei. Wyrobek *et al*. (1990) developed a technique for sperm isolation, nuclear decondensation, slide preparation and fluorescence *in situ* hybridization (FISH). Among several techniques used to detect aneuploidy since 1970 (Pearson and Bobrow, 1970), FISH is the most developed and applied method, especially during the last decade (Hassold and Hunt, 2001). Depending on the number of DNA probes used, FISH provides information on the degree of numerical chromosome aberrations. In practice, this is usually limited to the detection of up to four chromosomes (Downie *et al*., 1997a,b). Hassold and Hunt (2001) have estimated that aneuploidy occurs in at least 5% of all clinically recognized pregnancies. Martin and Rademaker (1990) showed that aneuploidy in the sex chromosomes is caused by paternal meiotic error more commonly than aneuploidy in the autosomes, whereas Robbins *et al*. (1995) using three-probe FISH found that paternal age increased sex chromosome disomy. Germinal aneuploidies are a major cause of pregnancy loss, developmental defects and aneuploid births (Sloter *et al*., 2000). Numerical aberrations in the sex chromosomes can result in offspring having problems with fertility and normal sexual development [e.g. Klinefelters syndrome (47,XXY) and Turners syndrome (45,X); Buwe *et al*., 2005]. In addition to age, other exposures that have been correlated in more than one study to the risk for human sperm aneuploidy include alcohol use, cigarette smoking and exposure to ionizing radiation, chemotherapeutic agents and air pollution.

Upon initial review of the literature, it was apparent that several studies evaluating pesticide health effects had converged around specific sperm endpoints and that the scientific literature was substantial enough to be systematically reviewed and summarized so that recommendations for future research directions could be made. A meta analysis to compute aggregate effect sizes across studies was not possible however because the measures of association used varied widely among the studies.

Materials and Methods

Search design

The scientific literature published between 1991 and 2006 was searched. This period was chosen to reflect findings over the past 15 years during which new technical applications have emerged for measuring exposures and health effects in reproductive, occupational and environmental epidemiology studies.

Identification of studies

Studies were identified mainly by using Medline databases. Handsearch was a second search method used to explore the references of retrieved articles. Systematic searches of the scientific literature were conducted using the following key words: human sperm, DNA fragmentation, DNA damage, sperm quality, semen parameters, spermatogenic effects, sperm aneuploidy, chromosomes, sex chromosomes, fluorescence *in situ* hybridization, FISH or genotoxic effects in combination with any of the following words: pesticides, agrichemicals, occupational pesticide exposure, environmental pesticide exposure. Articles were limited to studies in humans and to reports published in English. Meeting proceedings were not included.

Eligibility criteria

All reports pertaining to pesticides and human sperm published in English were identified and screened. Only original research articles meeting the following eligibility criteria were included in the final search results: studies that investigated semen quality parameters or markers of DNA damage in sperm or aneuploidy or diploidy in human sperm cells using FISH; and that included men who were environmentally or occupationally exposed to any type of pesticide;

and that were published from 1991 to 2006. The following studies were also excluded to focus the scope of the review: studies investigating agricultural occupation alone as the exposure of interest without specifying pesticides; studies investigating polychlorinated biphenyls (PCBs) alone not including the pesticide dichlorodiphenyltrichloroethane (DDT); and studies focusing on sex chromosome ratio in sperm as the only outcome of interest.

Results

Fifty-eight (58) reports published after 1990 were identified that pertained to pesticides and human sperm. Using the eligibility criteria described previously, the following were excluded: 16 literature reviews, 3 studies investigating agricultural occupation alone without specifying pesticides, 5 studies investigating PCBs alone without including DDT and 2 sperm sex chromosome ratio studies. Thirty (30) reports concerned original studies of pesticides and sperm that were included in the review. The studies were divided into three separate categories and corresponding tables according to the primary outcomes assessed, Table I: semen quality (i.e. concentration, motility and morphology—20 studies); Table II: DNA damage (i.e. DNA fragmentation and comet tail DNA damage—6 studies); and Table III: numerical chromosome aberrations (i.e. disomy and aneupolidy—6 studies). Two studies evaluated both sperm quality and DNA damage in relation to pesticide exposures (Larsen *et al*., 1998; Sanchez-Pena *et al*., 2004) and were included in both Tables I and II, we therefore refer to a total of 32 studies see below (30 published reports).

Semen quality

Table I shows the 20 studies evaluating pesticide exposure and semen quality parameters and Supplementary Table I shows further detail.

Studies reporting no association

Three of 20 studies reported no association between pesticide exposure and semen quality indicators. Of these three, two studied occupational exposure and one studied environmental exposure. Sample sizes ranged from 25 men in the case group with male factor subfertility (Magnusdottir *et al*., 2005) to a cross-section of 33 pest sprayers (Sanchez-Pena *et al*., 2004). One study looked at biologically non-persistent herbicides, insecticides and fungicides (Tielemans *et al*., 1999); one looked at organochlorines (Magnusdottir *et al*., 2005); and one study measured both non-persistent pesticides and organochlorine exposures (Sanchez-Pena *et al*., 2004). One of the studies reporting no association relied on self-reported exposure (Tielemans *et al*., 1999). Sanchez-Pena *et al*. (2004) did not find an association between urinary dialkylphosphates [organophosphate (OP) metabolites] and any semen parameters analyzed including morphology, seminal volume, motility, sperm concentration and viability. Magnusdottir *et al*. (2005) did not find an association between serum *p,p*'-DDE and male factor subfertility based on poor semen quality defined as requiring two or more of the following: sperm concentration $\leq 10 \times 10^6$/ml and/or total sperm count $\leq 20 \times 10^6$ and/or progressive sperm motility $\leq 30\%$ and no marked clinical or pathological disorders. All three of the no association studies evaluated sperm quality based on two or more indicators of concentration, count, morphology and/or motility.

Studies reporting an association

Studies reporting associations varied widely in methods, exposures and outcomes. Thirteen of 20 (65%) studies reported an association between pesticide exposure and semen quality indicators, specifically sperm concentration (six studies), motility (six studies) and morphology (four studies). Five of the seven (71%) studies that relied on self-reported exposure, and 8 of the 13 (62%) studies that used chemical analysis of exposure, modeled both categorically and continually, showed associations. Associations were found across all pesticide classes including DDT (Dalvie *et al*., 2004), a composite score of organochlorines including *p,p*'-DDE and *p,p*'-DDT (Dallinga *et al*., 2002), OPs (Padungtod *et al*., 2000; Meeker *et al*., 2004a) and mixtures of other biologically non-persistent herbicides, fungicides and insecticides (Lerda and Rizzi, 1991; Swan *et al*., 2003; Lifeng *et al*., 2006).

Owing to use of similar methods, it was possible to compare across three studies reporting pesticide exposure levels in relation to sperm concentration. Lerda and Rizzi (1991) showed 2,4-D exposed sprayers (mean 9.02 mg/l urine) had lower sperm concentration: 49.0×10^6 versus 101.6×10^6 ml in non-exposed men. Padungtod *et al*. (2000) also showed OP exposed men (*p*-nitrophenol levels = 0.22 mg/l urine) had lower sperm concentration: 43.0×10^6 versus 75×10^6 ml in non-exposed men. Lifeng *et al*. (2006) also showed that fenvalerate exposed men (geometric mean for area sampling—21.6×10^{-4} mg/m^3) had lower sperm concentration: 54.0×10^6 versus 89.5×10^6 ml in non-exposed men.

DNA damage

Table II shows the six studies published between 1991 and 2006 evaluating associations between occupational or environmental pesticide exposure and DNA damage in sperm. Supplementary Table II shows further detail.

Studies reporting no association

One of the six studies examining pesticide exposure and sperm DNA damage did not report an association in Inuit men (Spano *et al*., 2005). This study, evaluating SCSA among men exposed to organochlorines found an association for some European men exposed to PCBs, measured as CB-153 unlike the Inuit men. However, there was no association for the organocholorine pesticide *p,p*'-DDE and fragmentation among any of the men studied.

Studies reporting an association

Three of six studies reported associations between environmental or occupational pesticide exposure and DNA damage. Using area monitoring in a pesticide manufacturing plant, Bian *et al*. (2004) reported that tail DNA damage (measured using the comet assay) and DNA fragmentation (measured using the TUNEL assay) were both higher in the fenvalerate exposure group compared with the low and no exposure groups. Sanchez-Pena *et al*. (2004) also reported that occupational exposure to OPs as evidenced by diakylphosphate urinary metabolites was associated with DNA fragmentation measured using SCSA. In the only environmental exposure study showing an association, Meeker

Table I. Summary of findings from studies on occupational/environmental pesticide exposure and semen quality.

First author (year)	Subjects	Pesticides	Effects assessed
Multiple herbicides, insecticides and/or fungicides			
Bigelow (1998)	55 exposed; 319 unexposed	Unspecified pesticides	Mean semen volume (4.5 versus 3.3 ml) & tapering head defects significantly higher in exposed group
Larsen (1998)	161 farm sprayers; 87 non-sprayers	Hs, Is, Fs	Non-significant changes found in sperm morphology, vitality & motility between 2 groups
Tielemans (1999)	43 exposed; 856 unexposed	Hs, Is, Fs	Exposure to pesticides was not associated with changes in semen quality
Abell (2000)	13 high, 64 intermediate, 44 low exposure	>60 pesticides: Hs, Is, Fs	Sperm concentration (36 versus 87 × 10^6/ml) & proportion normal spermatozoa (61% versus 71%) significantly lower in high exposure group
Padungtod (2000)	32 OP exposed workers; 43 controls	Is & OPs	Significant reductions in sperm concentration (mean = 43 versus 75 × 10^6/ml) & % motility (47 versus 57) in high exposure group
Oliva (2001)	40 exposed, 80 non-exposed	Hs, Is, Fs	Exposure associated with increased seminal volume (OR 2.8, CI 1.2–6.6); & lower sperm concentration (OR 3.0, CI 1.2–7.4) output (OR 2.7, CI 1.1–6.7) & motility (OR 4.5, CI 1.8–11.5)
Swan (2003)	50 low semen parameters; 36 within normal limits	Al, IMPY, At, M, 2,4-D & others	Odds for low semen quality higher in men exposed to Al (OR 30.0, CI 4.3–210) At (OR 11.3, CI 1.3–98.9) & IMPY (OR 16.7, CI 2.8–98.0)
Kamijima (2004)	18 pesticide sprayers; 18 controls	Is	Percent of slow progressive (15.6 versus 8.8) & non-progressive motile sperm (5.9 versus 2.5) twice as high in the sprayers spraying in summer
Sanchez-Pena (2004)	33 men selected from 227 workers	Hs, Is, Fs	No significant association between semen quality & DETP or DAP
Yucra (2006)	31 pesticide sprayers; 80 unexposed	OPs	Sprayers had significantly lower seminal volume (2.1 versus 2.7 ml), percentage motility (58.6 versus 71.0), percent normal morphology grade A (18.7 versus 28.3) & grade A+B (53.6 versus 64.2)
Single pesticides			
Lerda (1991)	32 farm sprayers; 25 controls	2,4-D	Farm sprayers had significantly lower semen concentration: 49.0 versus 101.6 × 10^6/ml in controls
Meeker (2004a)	272 recruited from an infertility clinic	Carbaryl, Chlorpyrifos	1N associated with lower sperm concentration (OR 4.2, CI 1.4–12.6) & motility (OR 2.4, CI 1.2–4.5)
Lifeng (2006)	32 pesticide plant workers; 68 non-exposed controls	Fenvalerate	Sperm count significantly lower in exposed group (GM = 54.0 versus 89.5 10^6/ml)
DDT and metabolites			
Dallinga (2002)	31 with normal PMSC; 34 men below normal PMSC	PCBs, HCB, *p,p'*-DDE & *p,p'*-DDT	Correlations between OC metabolites in blood & sperm count ($R^2 = 0.14$; $P = 0.04$) & PMSC ($R^2 = 0.17$; $P = 0.02$) in normal semen quality group
Hauser (2002)	29 cases; 18 men with normal semen parameters	PCBs & *p,p'*-DDE	General trends of an association between PCBs & *p,p'*-DDE & abnormal motility, sperm concentration & morphology; no statistical analyses due to small sample size
Hauser (2003)	212 partners of subfertile couples	PCBs, *p,p'*-DDE	Significant association for PCBs but limited evidence of association between *p,p'*-DDE & motility
Dalvie (2004)	27 unexposed; 27 highly-exposed	DDT	Serum *p,p'*-DDT negatively associated with sperm count 10^6/ml (adjusted $R^2 = 0.05$ $P = 0.04$)
Pant (2004)	45 fertile & 45 infertile men	HCH & DDT	High levels of pesticides observed in semen of infertile men
Rignell-Hydbom (2004)	195 Swedish fishermen	*p,p'*-DDE, CB-153	Inverse but non statistically significant association between serum levels of CB-153 & sperm motility
Magnusdottir (2005)	25 with subfertility; 47 with normal semen quality	PCBs, *p,p'*-DDE	No difference in the level of OCs between the groups

Hs = Herbicides; Is = Insecticides; Fs = Fungicides; OR = odds ratio; CI = 95% confidence interval; Al = Alachlor; IMPY = 2-isopropoxy-4-methyl-pyrimidinol; At = Atrazine; M = Metolachlor; 2,4–D = 2,4-dichlorophenoxyacetic acid; DETP = Diethylthiophosphate; DAP = dialkyphosphates; OPs = organophosphates; 1N = 1-naphthol; GM = geometric mean; PMSC = Progressively motile sperm concentration; DDT = Dichlorodiphenyltrichloroethane; PCB = polychlorinated biphenyl; HCB = hexachlorobenzene; *p,p'*-DDE = 1,1-dichloro-2,2-bis (*p*-chlorophenyl-ethylene); *p,p'*-DDT = 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethylene;CB-153 = 2,2'4,4'5,5'-hexachlorobiphenyl; OCs = Organochlorines.

et al. (2004b) found urinary metabolites of carbaryl and chlorpyrifos were associated with DNA integrity measured using the neutral comet assay.

It was difficult to directly compare effect sizes across these three significant studies because they each used different indicators of DNA damage and/or ways of quantifying exposures. Bian *et al*. (2004) and Meeker *et al*. (2004b) both evaluated total comet tail DNA (expressed as a percentage of the total fluorescence measured in each comet tail during image analysis) using the comet assay however one study modeled exposure dichotomously and the other modeled exposures in inter-quartile ranges (IQR). Mean percentage comet tail DNA among 260 fertility clinic patients in the Meeker study was 26.5. An IQR increase in the chlorpyrifos metabolite TCPY was associated with a 2.76% comet tail DNA increase. An IQR increase in the carbaryl metabolite 1-napthol was associated with a 4.13% comet tail DNA increase. By comparison, Bian *et al*. reported 11.3% comet tail DNA in 21 men exposed to fenvalerate which was significantly different from 5.6% comet tail DNA in 23 non-exposed men.

Table II. Summary of findings from studies on occupational/environmental pesticide exposure and sperm DNA damage.

First author (year)	Subjects	Pesticides	Effects assessed
Multiple herbicides, insecticides and/or fungicides			
Larsen (1998)	161 farm sprayers; 87 farmers not spraying pesticides	Hs, Is and Fs	Significant decrease in SCSA parameter pre- and post-season (−1.7 median percentile) compared to an increase in SCSA parameter in non-sprayers (2.5 median percentile); however difference was within range of interassay variation
Sanchez-Pena (2004)	33 men selected from 227 workers	OPs 49%; Fs 19%; Hs 3%; C 5%; P 5%; BPs 3.8%; OCs 1.3%; Others 10.6%	Urinary DETP significantly associated with mean DNA fragmentation index ($P = 0.03$)
Single pesticides			
Bian (2004)	21 pesticide plant workers; 23 non-exposed clerical workers; 19 workers from local board of health	Fenvalerate	Median % comet tail DNA (11.3 versus 5.6) and olive tail moment (3.8 versus 1.5) was significantly higher in plant worker group than the clerical worker group
Meeker (2004b)	260 men from couples seeking infertility diagnosis at an infertility clinic	Carbaryl, Chlorpyrifos	Significant increase in % comet tail DNA for IQR increase in carbaryl (OR 4.1, CI 1.9–6.3) and chlorpyirfos (OR 2.8, CI 0.9–4.6)
DDT and metabolites			
Rignell-Hydbom (2004)	176 Swedish fishermen	*p,p'*-DDE CB-153	A significant association between serum levels of CB-153 and DNA fragmentation, however, not statistically significant for *p,p'*-DDE
Spano (2005)	193 Inuit from Greenland; 178 Swedish fishermen; 141 men from Warsaw, Poland; 195 men from Ukraine	CB-153; *p,p'*-DDE	Positive association between CB-153 and fragmentation in European men; No association between CB-153 and Inuit men or *p,p'*-DDE and fragmentation

Hs = Herbicides; Is = Insecticides; Fs = Fungicides; SCSA = sperm chromatin structure assay; OPs = Organophosphates; C = Carbamates; *P* = Pyrethroids; BPs = Biological pesticides; OCs = Organochlorines; DETP = Diethylthiophosphate; IQR = Inter-quartile range; OR = Odds ratio; CI = 95% confidence interval; *p,p'*-DDE = 1,1-dichloro-2,2-bis (*p*-chlorophenyl-ethylene); CB-153 = 2,2'4,4'5,5'-hexachlorobiphenyl.

Numerical chromosome aberrations

Table III shows the six studies published between 1991 and 2006 evaluating evidence for an association between occupational or environmental pesticide exposure and numerical chromosome aberrations. Supplementary Table III shows further detail.

Studies reporting no association

Harkonen *et al*. (1999), investigating diploidy in chromosomes 1 and 7, showed that occupational exposures of 32 Danish farmers to insecticides, herbicides and fungicides were not associated with sperm diploidy, but found that smoking significantly increased frequencies of sperm disomy 1-1-7 and sperm diploidy 1-1-7-7 after controlling for age, alcohol intake and sperm concentration. They also found that age was negatively associated with disomic cells for 1-7-7 ($P = 0.03$) before exposure, but not after exposure. They posited that chemicals contained in cigarettes were capable of inducing aneuploidy and diploidy in sperm cells by affecting meiotic segregation, although after adjustment for confounding exposures the cigarette association was found to be borderline significant for disomy 1-1-7 ($P = 0.06$). Smith *et al*. (2004) found that environmental exposures to insecticides, herbicides and fungicides were not associated with any aneuploidy for chromosomes 13, 21, X and Y and showed that the frequencies of sex chromosome diploidy were slightly lower in cases (Table III). Contrary to the Harkonen *et al*. (1999) finding, they did not find a smoking effect. It is difficult to directly compare the findings from Harkonen *et al*. (1999) and Smith *et al*. (2004) because they studied different chromosomes, they focused on different pesticides and they assessed different kinds of exposure (i.e. occupational versus environmental exposure).

Studies reporting an association

Four studies (Padungtod *et al*., 1999; Recio *et al*., 2001; Xia *et al*., 2004, 2005) investigating the frequency of numerical aberrations in chromosomes X, Y and 18 found positive associations between occupational pesticide exposure and aneuploid sperm cells (Table III). All four studies assessed only occupational exposures to pesticides, but they used different sample sizes and studied different pesticides. Padungtod *et al*. (1999) and Recio *et al*. (2001) assessed exposure to OP pesticide mixtures, whereas Xia *et al*. (2004) investigated exposure to fenvalerate, a pyrethroid insecticide and carbaryl, a carbamate insecticide (Xia *et al*., 2005). In the Padungtod *et al*. (1999) and the Recio *et al*. (2001) studies, the numbers of exposed cases were small, 13 and 4, respectively, which limited statistical power. The Recio *et al*. (2001) data suggested a slight association (Table III). Their results showed a positive association between urinary OP metabolites and sex chromosome nullisomy and total sex chromosome aneuploidy frequencies even after controlling for lifestyle factors and age.

Discussion

Semen quality studies: weight of evidence and biological plausibility

It was possible to compare across three well designed studies that used chemical analysis to isolate and quantify specific pesticide

Table III. Summary of findings from studies on occupational/environmental pesticide exposure and sperm aneuploidy (disomy and nullisomy) and/or diploidy detected by FISH.

First author (year)	Subjects	Pesticides	Effects assessed
Multiple herbicides, insecticides and/or fungicides			
Harkonen (1999)	30 healthy farmers selected before and after exposure	83 different types of pesticides including dithio-carbamate and morpholine fungicides	Exposures were not significantly associated with aneuploidy. Mean frequency for diploidy 1-1-7-7 was 0.11% before exposure and 0.09% after exposure to pesticides
Padungtod (1999)	13 workers from a large pesticide plant and 16 controls from a nearby textile factory	OP, Ethyl- and methyl parathion, methamidophos	Crude frequencies of total aneuploidies were 0.30% for exposed and 0.19% unexposed men. Rate ratio for aneuploidy among the exposed group was 1.56 (CI 1.1–2.3)
Recio (2001)	4 pesticide sprayers and 5 non-sprayers	OPs including methyl parathion, methamidophos, endosulfan, dimethoate	Most frequent aneuploidy was sex null (0.19%), followed by diploidy XY18-18 (0.06%). Total aneuploidies in X, Y & 18 were significantly higher (0.72%) during spraying compared to before spraying (0.59%)
Smith (2004)	20 exposed; 20 non-exposed	Is, Hs, Fs	Diploidy frequencies for sex chromosomes were not significantly different in exposed group (0.16%) compared to the non-exposed group (0.28%)
Single pesticides			
Xia (2004)	12 exposed workers, 12 internal controls and 18 external controls	Fenvalerate	Frequency of sex chromosome disomy was significantly associated with exposure: 0.74% in exposed group; 0.56% in internal control group and 0.37% in external control group
Xia (2005)	16 exposed workers, 12 internal controls and 18 external controls	Cabaryl	Frequency of sex chromosome disomy was significantly associated with exposure: 0.66% in exposed group; 0.56% in internal control group and 0.39% in external control group

OPs = Organophosphates; CI = 95% confidence interval; Is = Insecticides; Hs = Herbicides; Fs = Fungicides.

exposures and that measured differences in sperm concentration. These studies reported significant net decreases in sperm concentration in the exposure groups ranging from 39% for fenvalerate exposure (Lifeng *et al*., 2006) to 51% for 2,4-D (Lerda and Rizzi, 1991). Fenvalerate is a pyrethroid insecticide and prior work has shown that this chemical class has estrogenic activity, particularly in its metabolite forms (Tyler *et al*., 2000), and can cause sexual dysfunction in male rats (Ratnasooriya *et al*., 2002). However, the Lerda and Rizzi study was the first and remains the only study to report that the phenoxyacetic herbicide 2,4-D can act as a human male reproductive toxicant by affecting the germinal epithelium. Mammalian studies conducted to date have yet to demonstrate compelling evidence that 2,4-D is a reproductive toxicant and biological mechanisms of action on the human reproductive system have not been well elucidated.

Some of the organochlorine studies involved better exposure assessment precision by separating out specific isomers and metabolites of DDT. For this chemical class, the form of the compound is particularly important because DDE and DDD are degradation and metabolic products of DDT and humans are usually exposed to a mixture of these three compounds. Technical grade DDT typically consists of 77% *p,p*'-DDT, 15% *o,p*'-DDT, 4% *p,p*'-DDE and less than 1% *o,p*'-DDE, *p,p*'-DDD and o,p'-DDD. Results of *in vitro* and *in vivo* rodent studies suggest the *o,p*' isomers can act as estrogen agonists, whereas the *p,p*' isomers have androgen antagonist activity [Agency for Toxic Substances and Disease Registry (ATSDR), 2002]. Of the studies evaluating *p,p*'-DDE specifically, one study found no association (Magnusdottir *et al*., 2005) and three others found suggestive but non-significant ($P > 0.05$) associations. Only one study reported a significant association; however, in this study, *p,p*'-DDE exposure was not evaluated separately, and was included in a sum of other organochlorines including PCBs, hexachlorobenzene and *p,p*'-DDT. Two studies measured exposure to the parent compound DDT and both reported inverse associations with semen quality parameters (Dalvie *et al*., 2004; Pant *et al*., 2004). The weight of evidence thus far is stronger for total DDT than for its isomeric or metabolite forms.

DNA damage studies: patterns by chemical class

DNA damage in sperm is thought to be caused by incomplete maturation during spermiogenesis and apoptosis (Sakkas *et al*., 1999). Of the two studies using the comet assay to measure percent comet tail DNA, one study found an association with the pyrethroid fenvalerate (Bian *et al*., 2004), whereas the other found associations with the carbamate carbaryl and the organophospate chlorpyrifos (Meeker *et al*., 2004b). Pyrethroids are known to disrupt mammalian and amphibian germ cell formation, whereas the testicular toxicity of carbaryl has been demonstrated in rats (Pant *et al*., 1996). One of the four studies using SCSA found associations between the OP metabolite diethlythiophosphate and DNA fragmentation index (Sanchez-Pena *et al*., 2004). OPs are considered potent alkylating agents and alkylating agents are potentially genotoxic to animal sperm by altering chromatin structure via binding to protamines and DNA, causing DNA to become more susceptible to induce denaturation *in situ* (Evenson *et al*., 1985). Thus, three of the six DNA damage studies reported associations with chemical classes previously shown to be testicular toxicants in animals, substantiating the biological plausibility of these associations.

Numerical chromosome studies: common features

Numerical chromosomal aberrations in sperm are due to non-disjunctional events during meiosis; however, the causes of non-disjunction are not known. Although a limited number of studies have been conducted to determine if pesticide exposures can have aneugenic effects, some discernable patterns emerged from the six studies conducted to date. The four studies reporting a positive association all: (i) studied occupational exposures, and three of the studies were in pesticide manufacturers; (ii) utilized exposure assessment methods beyond self-report; (iii) found an association for the sex chromosomes; and (iv) found an association for total aneuploidies or disomy, but not diploidy. OPs, fenvalerate and carbaryl exposure were all associated with increased numerical aberrations and both the genotoxic and hormone disruptiong properties of these compounds have been established in animal studies. It is noteworthy that none of the numerical chromosome aberration studies focused on organochlorine exposures.

In total, 20 of the 32 studies reviewed reported associations between pesticide exposures and sperm production; however, not all studies were equally rigorous in their designs. Using study design quality (i.e. sample size, exposure assessment and standard outcome measures) and biological plausibility (i.e. the pesticide has previously demonstrated reproductive toxicity or endocrine altering properties) to evaluate the weight of evidence across all 32 studies, the insecticides carbaryl and fenvalerate emerge from five different studies as being consistently associated with sperm quality, DNA damage and numerical chromosomal aberrations. Clearly these insecticides deserve further research attention. Taking the findings of the 32 studies together, several study design recommendations for advancing this area of investigation can also be made.

Sample size

Sample sizes varied widely across the 32 studies and few if any of the studies provided information as to whether the study was adequately powered to detect the effect sizes investigated. The six studies investigating aneuploidy in particular had small sample sizes, with a range of 4–30 and a median of 16. Applying strict statistical power criteria illustrates the potential pitfall of conducting underpowered studies. Strictly speaking, using a background total aneuploidy rate of 2% for example, with 80% power to detect a 2-fold difference between the exposed and unexposed groups, a sample size of at least 90 in each group would be necessary. In planning the next set of studies investigating the spermatogenic effects of pesticides, it will be important to take the desired statistical power, effect size and background prevalence of the outcome of interest into account so that an adequate sample size is achieved.

Methods of exposure assessment

A third of the studies utilized only self-reported exposure to pesticides, which increases the possibility of exposure misclassification in the exposure and/or control groups. It appears that chemical exposure analysis has increasingly been used over the last 15 years, and should become a standard feature in future studies. To quantify exposure, future studies should strive to be as comprehensive as is feasible by using a combination of air monitoring, personal dosimetry and biomonitoring in urine or serum, depending on the pesticide compounds of interest. Whenever possible, studies should be designed to provide effect estimates for chemical mixtures due to potential synergistic effects (Perry *et al*., 2007).

Exposure timing also needs more consideration in future study designs. The various pesticides may act via different mechanisms, e.g. as hormone agonists or antagonists, as epididymal toxicants destroying specific cell types, or as germ cell mutagens causing production of sperm unable to fertilize. Effects on early stages are detectable in the ejaculate after a delay of 2–3 months, whereas effects of agents acting on the late stages of spermatogenesis or on epididymal function may show up in the ejaculate after a few days of exposure, if not immediately (Larsen *et al*., 1998).

Previous studies have not adequately addressed exposure timing because critical windows of chemical insult in the human spermatogenic cycle are not well known. In human studies of cytotoxic therapies, for example, the sensitivity of the testis to cytotoxic therapies that decrease sperm numbers is proportional to the proliferation of these cells (Meistrich, 1986). Of the germ cells, spermatogonia are the most proliferative and are most susceptible to apoptosis induced by cytotoxic therapy. Spermatocytes and spermatids are insensitive to cytotoxic agents, which is evidenced by the maintenance of normal sperm counts for the first 2 months of cytotoxic therapy. After 2–3 months of receiving therapy, counts decrease significantly, indicative of the effects on spermatogonia, which in the 2–3 month interval have become spermatozoa (Chapman *et al*., 1981). If stem cells survive and differentiate, sperm production can recur, however this takes 1–2 years after cytotoxic insult (Clifton and Bremner, 1983). Patients receiving chemotherapeutic agents showed elevated aneuploidy of autosomal and sex chromosomes in sperm, but the effects were transient and declined to pretreatment levels ~100 days after treatment (Robbins *et al*., 1997). Risks for chromosomal damage were highest within one spermatogenic cycle (3 months) after the male was exposed to cytotoxic agents. Unique critical windows or stages of human sperm production most vulnerable to exogenous exposures are not well understood, however both pre- and post-meiotic cells appear vulnerable to chemical injury and more information on stage specific effects is needed. Future studies should seek to determine exposure timing and, when feasible, include pre-exposure sperm samples for analysis.

Confounding

Of concern is the extent to which studies were able to uniformly control for confounders such as age and abstinence time, and ensure adequate quality control in sperm parameter assessment, which is inherently vulnerable to intra and interlaboratory variability (Cooper *et al*., 2002). Age, smoking and abstinence time are factors previously associated with sperm quality and DNA integrity, and age and smoking has been associated with aneuploidy risk. These factors may act as confounders if they also affect the circumstances of pesticide exposure. Most of the sperm quality studies controlled for abstinence time either by instructions to participants prior to sample collection or as a statistical covariate. However, only a portion of the 32 studies reviewed here were successfully able to control for age and smoking. Future studies should set as standard the control for abstinence time, age, smoking, alcohol use, because all can potentially act as confounders. Medical history including prior testicular disease, other medical causes of infertility or inferior sperm quality, and medication use should also be assessed as potentially important effect modifiers. Similarly, standard quality control techniques including blinded study designs and replicate scoring should be routinely included when feasible to reduce differences attributable to interlaboratory variability. Because field studies requiring semen collection are intrusive, the extent to which volunteer bias impacts the inclusion of study participants should be routinely evaluated by tracking refusal characteristics and overall response rates.

Deciding on outcomes of interest

Each of the sperm outcomes considered here, sperm quality, DNA damage and aneuploidy, warrant increased attention because their reproductive consequences are important, they can be reliably assessed using well established protocols and assays, and environmental causes cannot be ruled out. The pattern emerging from the sperm parameter studies was that sperm concentration was lower in pesticide exposed men and that pesticide exposure was most often associated with sex chromosome disomy in the numerical chromosome aberrations studies. The next phase of studies should incorporate each of these end-points in the same study. Several studies not examining environmental exposures (Rives *et al*., 1999; Hassold and Hunt, 2001; Shi and Martin, 2001; Rubes *et al*., 2002) have drawn attention to the relationship between semen quality and aneuploidy frequencies in sperm. Rubes *et al*. (2002) reported no consistent association between individual categories of aneuploidies and diploidies in sperm and sperm quality in healthy men, but did report a significant positive correlation between sex chromosome aneuploidies in sperm and in lymphocytes. Shi and Martin (2001) summarized that their and other studies have demonstrated an increased risk of XY and 21 disomy in men with low sperm concentration, but found conflicting evidence of a relationship between sperm morphology and the frequency of sperm aneuploidy. In the six aneuploidy studies reviewed here, three of them (Harkonen *et al*., 1999; Xia *et al*., 2004, 2005) explored the association between semen quality and aneuploidy in sperm. Xia *et al*. (2004) investigated the effect of fenvalerate and its metabolites on sperm morphology and found that morphologic abnormalities and spermatozoa defects were higher in exposed workers; however, they reported a lack of strong association between pesticide exposure and some conventional semen parameters such as progression and motion. This same group (Xia *et al*., 2005) also found an association between carbaryl exposure and sperm morphology but not other sperm quality indicators. Harkonen *et al*. (1999) found that sperm concentration had a significant negative association with diploid sperm cell frequency and with aggregate aneuploid sperm cell frequency after exposure. These associations were not observed in the same group before the fungicide spraying season. The status of the literature is suggestive enough to warrant expanding future studies to explore interrelationships across each sperm end-point.

The prospects of vulnerable subgroups also need to be considered in future study designs. In studying repeated semen specimens from healthy men, Rubes *et al*. (2002) reported a significant association between the frequencies of sex chromosome aneuploidies in sperm and lymphocytes, suggesting that apparently healthy men can produce significantly higher frequencies of both aneuploid sperm and lymphocytes. This finding needs further exploration in other samples of healthy men. Such a pattern may allude to constitutive genetic susceptibilities for increased aneuploidy frequencies which may affect both germ cells and somatic cells. If such susceptibilities do exist, it follows that there may be vulnerable subgroups particularly susceptible to segregation problems influenced by environmental/occupational chemical exposures such as pesticides. This finding suggests the need to evaluate semen quality, DNA damage and aneuploidy at baseline, prior to pesticide exposure, and again post-exposure. The prospects of vulnerable subgroups also reinforce the importance of including a well-characterized control group in studies investigating chromosomal patterns and potential pesticide exposure effects.

Conclusions

Human exposure to environmental and occupational chemicals has increased considerably in the past 50 years. Pesticides are among the most produced and used chemicals in the USA and internationally. Pesticides may operate through hormonal or genotoxic pathways to affect male reproduction. They may penetrate the blood testis barrier to potentially affect spermatogenesis, either by affecting genetic integrity or hormone production (Toppari *et al*., 1996). Effects may be at different stages of the cell cycle such as during meiotic disjunction, and such abnormalities can have deleterious effects on reproduction and offspring. As reviewed here, 20 epidemiologic studies have reported associations between pesticide exposures and sperm production. On the basis of this review, the evidence from pesticide and sperm parameter studies are suggestive, but not consistent enough to elucidate neither specific pesticides nor definitive sperm quality end-points. The sperm DNA damage and aneuploidy evidence accumulated thus far is not sufficient to support definitive effects. However, these studies elucidate some important patterns requiring closer investigation.

In an effort to achieve more specificity and replication in this field of inquiry, the next wave of studies investigating spermatotoxic effects of pesticides should attempt to: (i) measure the effects of specific individual chemical exposures, mixtures and potential synergies; (ii) use standard biomonitoring methods to isolate and quantify the compounds of interest in serum or urine; (iii) expand the sperm outcomes of interest in the same study to include sperm quality parameters, DNA damage markers and numerical chromosome aberrations so that comparisons can be made across end-points within the same studies; and (iv) ensure adequate control for confounders including age and abstinence time. Further human studies are necessary to clarify both the effects of current environmental or occupational pesticide exposures on male reproductive health and the physiologic mechanisms underlying these effects.

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Supplementary Data

Supplementary data are available at http://humupd.oxfordjournals.org/

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