EPIDEMIOLOGICAL STUDIES ON THE RELATIONSHIP BETWEEN SEMEN QUALITY AND ENVIRONMENTAL CHEMICALS: *HISTORIC AND CONTEMPORARY COMPOUNDS*

R. HAUSER[1] AND J.D. MEEKER[2]
[1] *Department of Environmental Health*
Harvard School of Public Health
Boston, MA 02115
USA
[2] *Department of Environmental Health Sciences*
University of Michigan
Ann Arbor, MI 48109
USA

Summary

Recent reports of downward trends in semen quality and increased rates of developmental urogenital tract anomalies and testicular cancer have raised both scientific and public concern about the potential risk of environmental chemicals to male reproductive health. Of particular concern is whether some contemporary use environmental chemicals alter semen quality. Semen quality refers to both conventional measures, such as sperm concentration, motility and morphology, as well as sperm DNA integrity as assessed by bioassays. Specific toxicants of interest include phthalates and pesticides (primarily insecticides and herbicides), as well as polychlorinated biphenyls (PCBs). The human data on the relationship of semen quality with phthalate and pesticide exposure are limited and does not currently allow for a definitive conclusion on whether adult exposure, at background environmental levels, alters semen quality. However, the epidemiologic data support an inverse association of PCBs with reduced semen quality, specifically reduced sperm motility. The associations found were generally consistent across studies despite a range of PCB levels. In addition to the chemicals discussed in detail, there are additional classes of chemicals that require further study as to their relation with

P. Nicolopoulou-Stamati et al., (eds.), Reproductive Health and the Environment, 23–56.

human semen quality. These chemicals include alkylphenols, such as 4-nonylphenol, bisphenol A and the fluorinated organic compounds.

1. Introduction

Scientific and public concern about the potential risk of environmental chemicals to male reproductive health has been driven by reports of temporal downward trends in semen quality (Carlsen *et al.,* 1992; Swan *et al.,* 2000), increased rates of development anomalies, specifically hypospadias and cryptorchidism (Paulozzi, 1999), and increased rates of testicular cancer (Adami *et al.,* 1994; Bergstrom *et al.,* 1996; Huyghe *et al.,* 2003). Furthermore, a recent study among healthy young men found an unexpectedly high proportion of poor semen quality (Andersen *et al.,* 2000). These observations raise the possibility that human exposure to environmental chemicals may partially be responsible.

The assessment of semen quantity and quality is used clinically to assess potential fertility (WHO, 1999) and in epidemiological studies as a biomarker for the potential effects of toxicants on the male reproductive system. Semen quantity and quality may be altered through toxicant affects on the neuroendocrine system (i.e., the hypothalamic-pituitary-testis axis), the testis (which includes Sertoli and Leydig cells as well as the spermatogenic cells), and on post-testicular sites such as the epididymis. Potential toxicants may affect semen quantity and quality by interacting with or disturbing one or more of these targets.

Although semen quality is measured in the adult male, it may be affected by exposures during various life stages, such as during gestation, puberty, or as an adult. In addition, as recently shown by an elegant study by Anway *et al.* (2005), there are transgenerational effects of chemicals, whereby exposure of the maternal or paternal (or even the grandparents) gametes to chemicals may confer an increased risk of altered semen quality in the offspring. Although early life exposure may impair spermatogenesis, as supported by evidence from studies in laboratory animals and human studies on prenatal exposure of men to DES, the human data is generally limited to the assessment of both semen quality and environmental or occupational exposure during adult life. Therefore, the present chapter largely describes evidence on the relationship between adult exposure to environmental chemicals and semen quality. It is anticipated that evidence on exposure during early life stages, such as gestation and puberty, will become available in the coming years.

In the present chapter, we focus on epidemiologic studies that explored the relationship of semen quality with several contemporary use environmental toxicants. Specific toxicants include the following: phthalates, pesticides (primarily the contemporary use non-persistent pesticides), bromopropane and polychlorinated biphenyls (PCBs). Although PCBs are not currently in use, we included PCBs in the chapter because there are many recent publications worthy of discussion.

The majority of epidemiologic studies on the environment and semen quality are cross-sectional designs in which exposure and semen parameters were assessed at a single point in time. This makes it difficult to assess causation because it is not always clear that exposure precedes outcome. In addition, the reversibility or permanence of the effect, if one is present, is also difficult, if not impossible, to determine. It is well-known that semen quality parameters, such as sperm concentration, motility and morphology, vary both between as well as within individuals. The variability in semen parameters may be related to biological and/or social factors (such as abstinence time). Because of the within individual variation in semen parameters, the collection of a single semen sample makes it difficult to assess the relationship between chemical exposures and human semen parameters. The intra-individual variability will generally tend to bias associations, if present, to the null hypothesis. Another limitation of most human semen quality studies is the low participation rates, often well below 50 per cent. This may introduce selection bias if men agree to participate based on knowledge of both exposure and outcome (semen quality). In addition, some epidemiologic studies may not collect information on important potential confounders and/or may not have adjusted for confounders.

Because this chapter is not an exhaustive review of the epidemiologic literature on environmental and occupational toxicants and semen quality, the reader is directed towards previously published reviews and book chapters. These earlier publications discuss known human male reproductive toxicants, primarily occupational agents, such as 1,2-dibromo-3-chloropropane (DBCP), diethylstilbestrol (DES), inorganic lead, alkylating neoplastic agents, ethylene glycol, monomethyl and monoethyl ethers, carbon disulphide, ethylene dibromide, and ionizing radiation (Rosenberg *et al.,* 1987; Bonde and Giwercman, 1995; Lahdetie, 1995; Tas *et al.,* 1996; Figa-Talamanca *et al.,* 2001; Pflieger-Bruss *et al.,* 2004; Vidaeff and Sever, 2005).

2. Non-persistent pesticides

The term "non-persistent pesticides" (also commonly called "contemporary-use pesticides") refers to chemical mixtures that are currently available to control insects (insecticides), weeds (herbicides), fungi (fungicides) or other pests (e.g.

rodenticides), as opposed to pesticides that have been banned from use in most countries (e.g. many of the formerly popular organochlorine pesticides such as DDT). Some common classes of non-persistent pesticides in use today include organophosphates, carbamates, and pyrethroids. Though environmentally non-persistent, due to the extensive use of pest control in these various settings a majority of the general population is exposed to some of the more widely used pesticides at low levels.

There are several epidemiologic studies on men exposed to non-persistent pesticides during agricultural work. A cross-sectional study on testicular function measured sperm concentration, motility, and morphology in 122 greenhouse workers defined as low, medium or highly exposed to more than a dozen pesticides (Abell *et al.*, 2000). Adjusting for abstinence time and other potential confounders, a higher proportion of abnormal sperm were found in the high exposure group compared to the group with low exposure. Lower median sperm concentration was also observed in workers with more than 10 years of work in the greenhouse compared to men with less than 5 years of experience. In a cross-sectional study on traditional and organic farmers, Juhler *et al.* (1999) investigated the relationship between dietary exposure to pesticides and semen quality. Estimating exposure through food frequency questionnaires and data from pesticide monitoring programs, the authors found that men with a lower intake of organic food had a lower proportion of normal shaped sperm according to the strict criteria (2.5% versus 3.7%, p-value = 0.003). However, organic food intake was not associated with the other 14 semen parameters measured in the study. Results in the study were adjusted for age, urogenital tract disease, spillage, abstinence time, smoking, and alcohol intake. Oliva *et al.* (2001) investigated the impact of environmental factors on infertility among 177 men in Argentina. Adjusting for age, BMI, abstinence time, income, health center, and smoking, a dose-related response was observed in (primary) infertile men occupationally exposed to pesticides. Significantly elevated odds ratios (95 per cent confidence intervals) were reported for sperm concentration (less than 1×10^6/mL; OR = 3.4 (1.2, 7.4)), motility (<50% motile; OR = 3.6 (1.1, 11.4)), and morphology (<30% normal; OR=4.1 (1.4, 12.0)) for men exposed to pesticides compared to occupationally non-exposed men. Conversely, in models adjusting for many of the same variables, Larsen *et al.* (1998) found only marginal differences among 15 semen quality parameters from Danish farmers who sprayed pesticides compared to farmers that did not spray pesticides. These studies show a possible association between pesticide exposure and human semen quality. However, the non-specific assessment of pesticide exposure makes it difficult to determine which pesticides, if any, were responsible for the observed effects.

Few studies have been conducted that provide information on specific chemicals or classes of non-persistent pesticides and altered testicular function. Padungtod *et al.* (2000) studied the relationship between occupational exposure to organophosphates (parathion and methamidophos) and testicular function among Chinese pesticide factory workers. They found a significant reduction in adjusted mean sperm concentration (28.5 vs. 49.4 million sperm/mL; p-value = 0.01), and percentage of motile sperm (64% vs. 74%; p-value = 0.03) in the 20 exposed workers as compared to the 23 unexposed workers. In a recent Japanese study, pesticide sprayers exposed primarily to organophosphates and pyrethroids showed spraying season-dependant reductions in motile sperm velocity measures compared to unexposed controls (Kamijima *et al.*, 2004).

Two publications reported the results from a study on a small cohort of men exposed to carbaryl (1-naphthyl methyl carbamate; commonly known as Sevin®) during the production and packaging of the insecticide (Whorton *et al.*, 1979; Wyrobek *et al.*, 1981). Although analyses using sperm counts as a continuous measure failed to find significant differences based on carbaryl exposure, the authors found a greater proportion of oligozoospermic men among the carbaryl workers as compared to the chemical workers (Whorton *et al.*, 1979). In a subsequent publication on the same cohort of carbaryl production workers, Wyrobek *et al.* (1981) studied the relationship between sperm shape abnormalities and carbaryl. Morphological analyses showed an elevated percent of abnormal sperm in carbaryl workers as compared to comparison subjects, which remained after stratifying on potential confounders such as smoking, medical history, or previous exposure to hazardous agents. The proportion of men defined as teratospermics (greater than 60% abnormal sperm) was higher among the carbaryl workers than in the comparison group (28.6% and 11.8%, respectively).

More recently, researchers have utilized urinary and serum biomarkers of pesticide exposure to explore associations with reduced semen quality. In a US study on the male partners of pregnant women, Swan *et al.* (2003) compared urinary levels of pesticide biomarkers in 34 men with sperm concentration, motility and morphology below the median (defined as cases) to 52 men with above median semen parameters (defined as controls). They found elevated odds ratios [OR (95% confidence interval)] for alachlor mercapturate [30.0 (4.3, 210)], 2-isopropoxy-4-methyl-pyrimidinol (IMPY; diazinon metabolite) [16.7 (2.8, 98)], atrazine mercapturate [11.3 (1.3, 99)], 1-naphthol (carbaryl and naphthalene metabolite) [2.7 (0.2, 34)] and 3,5,6-trichloro-2-pyridinol (TCPY; chlorpyrifos metabolite) [6.4 (0.5, 86)]. However, a small study size led to the wide confidence intervals that restrict interpretation of the study results.

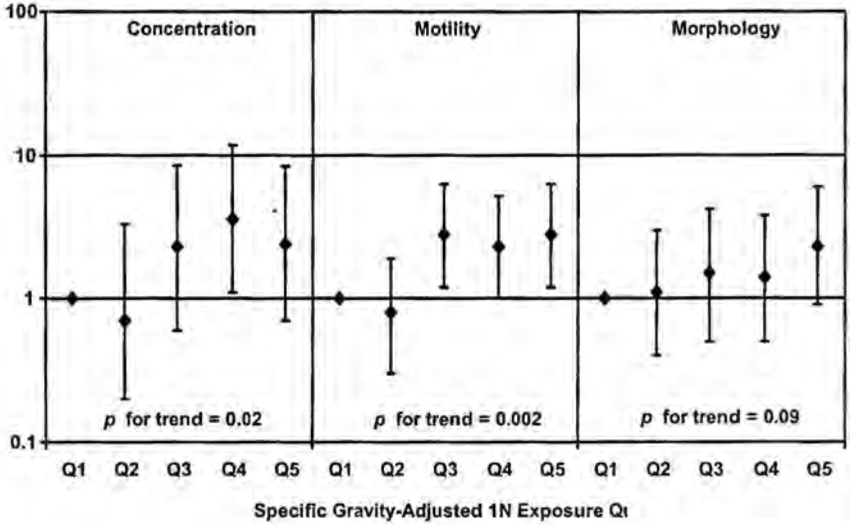

Figure 1. Odds ratios (95% confidence intervals) for the relationship between semen parameters and specific gravity-adjusted 1-naphthol (1N) exposure quintiles (from Meeker *et al.*, 2004a)

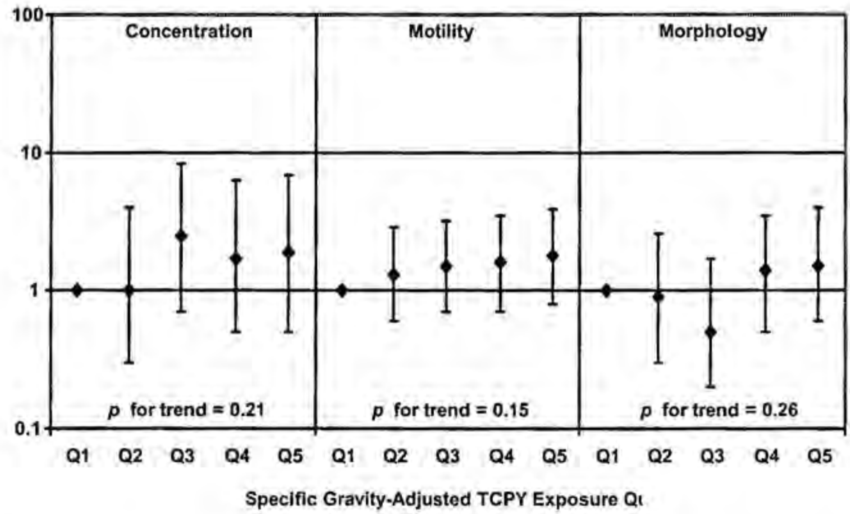

Figure 2. Odds ratios (95% confidence intervals) for the relationship between semen parameters and specific gravity-adjusted TCPY exposure quintiles (from Meeker *et al.*, 2004a)

Using urinary biomarker data representative of low environmental levels of pesticides commonly encountered among the general population, Meeker *et al.* (2004a) studied 272 men that were partners of an infertile couple. They found inverse associations between urinary levels of 1-naphthol, a metabolite of both carbaryl and naphthalene, with sperm concentration and motility (Figure 1). They also found a suggestive inverse relationship between the urinary metabolite of chlorpyrifos and sperm motility (Figure 2). When insecticide metabolite levels were categorized into tertiles, odds ratios (95% confidence interval) for medium and high tertiles of 1-napthol were 4.2 (1.4, 13.0) and 4.2 (1.4, 12.6) for below reference concentration (<20 million sperm/mL), and 2.5 (1.3, 4.7) and 2.4 (1.2, 4.5) for below reference motility (<50% motile sperm). In multiple linear regression analyses, an interquartile range increase in 1-naphthol (1.8 to 5.0 μg/L) was associated with a 3.9 per cent (-7.3, -0.5%) decline in proportion of motile sperm and a 16 per cent (-29, +1.0%) decline in sperm concentration. An interquartile range increase in chlorpyrifos metabolite (TCPY; also 1.8 to 5.0 μg/L) was associated with a 2.2 per cent (-5.1, +0.7%) decline in motile sperm.

Several studies have also explored the relationship between pesticide exposure and novel markers of male reproductive endpoints that extend beyond the conventional semen parameters. Recent human studies have reported increased sperm DNA damage associated with environmental exposure to carbaryl and the organophosphate chlorpyrifos (Meeker *et al.*, 2004b), and sperm genotoxicity following occupational exposure to carbaryl and the synthetic pyrethroid fenvalerate (Xia *et al.*, 2005; Bian *et al.*, 2004; Xia *et al.*, 2004). Additional studies have reported associations between organophosphate exposure and increased frequency of human sperm aneuploidy (Padungtod *et al.*, 1999; Recio *et al.*, 2001) and altered sperm chromatin structure (Sanchez-Pena *et al.*, 2004).

In summary, there are limited human studies supporting an association between altered semen quality and non-persistent pesticide exposure, specifically some herbicides and insecticides. However, the majority of studies were occupational studies involving simultaneous exposure to several pesticides. Thus, there is limited evidence on the relationship between male reproductive health endpoints and specific non-persistent pesticides. Furthermore, our understanding of low-level environmental exposure to non-persistent pesticides, primarily through diet and residential use, is not well understood. Additional research using biomarkers of exposure to specific pesticides is needed to further our understanding of the potential reproductive health risks associated with non-persistent pesticides.

Table 1. Summary table of epidemiologic studies (in chronological order) on the relationship between non-persistent pesticides and semen quality

Author, country	Study population	Exposure	Results	Comments
Whorton *et al.*, 1979 US	47 carbaryl production workers plus 90 unexposed controls	Subjective exposure classification based on job tasks	Greater proportion of oligozoospermic men among the carbaryl workers (15%) as compared to the chemical workers (5.5%; p-value=0.07).	No adjustment for potential confounders. Sperm motility not measured.
Wyrobek *et al.*, 1981 US	50 carbaryl production workers plus 34 unexposed controls	Exposure ranks/groups based on job type held for previous year	Elevated percent of abnormal sperm in carbaryl workers (52%) as compared to comparison subjects (42%; p-value<0.005). The proportion of men defined as teratospermics (greater than 60% abnormal sperm) was higher among the carbaryl workers (28.6%) than in the comparison group (11.8%; p-value=0.06).	Confounders considered: smoking, medical history, previous exposure to hazardous agents
Padungtod *et al.*, 2000 China	43 Pesticide factory workers; 20 high exposed and 23 with no or very low exposure	Occupational exposure to ethyl parathion and methami-dophos	Exposure associated with reduction in sperm concentration and motility, but not sperm morphology. Adjusted means for exposed and non-exposed workers were: 28.5 and 49.4 million sperm/mL (p-value=0.01), respectively, for sperm concentration; and 64% and 74% (p-value=0.03), respectively, for percentage of motile sperm.	Confounders considered: age, abstinence period, current smoking status.

Table 1. continued.

Author, country	Study population	Exposure	Results	Comments
Swan *et al.* 2003 US	86 male partners from couples attending prenatal clinic	Urinary levels of pesticides or metabolites (IMPY, 1N, TCPY, others)	Increased odds ratios (95% CI) for below reference semen parameters associated with high exposure group for alachlor mercapturate 30.0 (4.3, 210); IMPY 16.7 (2.8, 98); atrazine mercapturate 11.3 (1.3, 99); 1-naphthol 2.7 (0.2, 34); and TCPY 6.4 (0.5, 86).	Small study size limited statistical power; odds ratios were unadjusted for potential confounders.
Meeker *et al.*, 2004a US	272 male partners from couples attending infertility clinic	Urinary levels of insecticide metabolites (1N, TCPY)	Inverse association between urinary carbaryl metabolite (1N) and sperm concentration, motility. IQR increase in 1N associated with 16% decline in sperm concentration and 3.8% decline in motile sperm. Suggestive inverse association between chlorpyrifos metabolite (TCPY) and sperm motility.	Confounders considered: age, BMI, abstinence time, smoking status, race, season
Meeker *et al.*, 2004b US	214 men from couples attending infertility clinic	Urinary levels of insecticide metabolites (1N, TCPY)	Using the neutral comet assay to assess DNA damage in human sperm, found associations between urinary carbaryl and chlorpyrifos metabolites (1N, TCPY) with percentage of DNA in the comet tail (p-value=0.0003 and 0.004, respectively).	Confounders considered: age, BMI, abstinence time, smoking status, race, season

Table 1. continued

Author, country	Study population	Exposure	Results	Comments
Xia *et al.*, 2005	16 carbaryl-exposed workers and 30 controls	Men defined as exposed or unexposed based on job tasks and air monitoring	Men with high occupational exposure to carbaryl (>5 mg/m^3 according to air monitoring) had a higher percentage of sperm with fragmented DNA (21±9) compared to internal (13±12; p-value=0.04) and external (14±7; p-value=0.03) controls.	Several potential confounders considered for subject eligibility into study (health, age, smoking, alcohol), but not included in the models

Abbreviations: 2-isopropoxy-4-methyl-pyrimidinol [IMPY]; 1-naphthol [1N]; 3,5,6-trichloro-2-pyridinol [TCPY]; confidence interval [CI]; body mass index [BMI]

3. Solvents

Organic solvents are widely used for cleaning in industrial production processes and are also found in paint systems. Traditional solvents have long been used for the degreasing of metal, glass or plastic work pieces in electroplating facilities, paint shops, and assembly plants, while new solvents have been introduced over the last few decades for specialized applications in the military, aerospace, biotechnology, and computer/semiconductor industries (Burgess, 1995).

Of the limited human studies of solvent exposure and semen quality, a number of them involve occupational exposure to broad classes of solvents as opposed to specific chemicals. For example, a study among 1,152 male partners in couples recruited from two infertility clinics in the Netherlands found that occupational exposure to aromatic solvents, estimated through questionnaires and a job-exposure matrix, was associated with increased risk of abnormal semen parameters (Tielemans *et al.,* 1999). Likewise, an association between solvent exposure and increased risk of abnormal sperm motility and morphology was reported in a study among 177 men that were interviewed about prior occupational exposures when reporting to an Argentina infertility clinic (Oliva *et al.,* 2001). A third study among men recruited from Canadian infertility clinics found a dose-dependent increased risk in abnormal sperm motility associated with moderate and high exposure to organic solvents (Cherry *et al.,* 2001).

Human studies of specific occupational solvent exposure and negative impacts on semen quality are limited but have been reviewed previously (Figa-Talamanca *et al.*, 2001; Sheiner *et al.*, 2003). Associations have been reported for ethylene glycol ethers (Shih *et al.*, 2000; Veulemans *et al.*, 1993; Ratcliffe *et al.*, 1989), trichloroethylene (Chia *et al.*, 1996), styrene (Kolstad *et al.*, 1999), benzene, toluene and xylene (Xiao *et al.*, 2001). However, because new chemical formulations continue to be introduced in industry to fit specific process requirements, reproductive toxicology and epidemiology data are not extensive for many solvents currently in use. Therefore it is possible that data implicating specific chemicals in reduced semen quality and male reproductive health will emerge in the future.

A recent example of emerging human evidence for reproductive toxicity of a specific solvent is that of 2-bromopropane. 2-bromopropane is sometimes used as an intermediate in the synthesis of pharmaceutical dyes and other organic chemicals (Boekelheide *et al.*, 2004), though in the US is found primarily as an impurity in 1-bromopropane that is used in spray adhesives or as a degreaser. In Asia, occupational exposure to 2-bromopropane is more prevalent since it is also used as a substitute for ozone-depleting chlorofluorocarbons (CFCs). A study of both male and female workers in a South Korean electronics factory that were exposed to 2-bromopropane reported compelling, though not conclusive, evidence of reproductive toxicity (Kim *et al.*, 1996). Of 8 male workers exposed to 2-bromopropane, 2 were azoospermic and 4 others had sperm concentration of less than 20 million/mL or less than 50% motile sperm. None of the 12 unexposed comparison workers from the same plant had abnormal semen measures. Follow-up studies in animals showed that 2-bromopropane led to impaired spermatogenesis (Ichihara *et al.*, 1997; Takeuchi *et al.*, 1997). Conversely, a subsequent study by Ichihara *et al.* (1999) among workers from a 2-bromopropane factory did not find an association between exposure and semen quality. However, the study had limited statistical power and exposure monitoring among the workers revealed few samples with exposure levels above those experienced by the reference group. Though the human and animal evidence was deemed sufficient by an expert panel convened by the National Toxicology Program to show male reproductive toxicity of 2-bromopropane (Boekelheide *et al.*, 2004), additional human studies investigating 2-bromopropane exposure and semen quality are needed.

4. Phthalates

The diesters of 1,2-benzenedicarboxylic acid (phthalic acid), commonly known as phthalates, are a group of man-made chemicals with a wide spectrum of industrial applications. High molecular weight phthalates (e.g., di(2-ethylhexyl) phthalate [DEHP], di-isononyl phthalate [DiNP], di-n-octyl phthalate [DnOP]), are primarily used as plasticizers in the manufacture of flexible vinyl which, in turn, is used in consumer products, flooring and wall coverings, food contact applications, and medical devices (ATSDR, 1997; 2002; David *et al.*, 2001). Manufacturers use low molecular weight phthalates (e.g., diethyl phthalate [DEP] and dibutyl phthalate [DBP]) in personal-care products (e.g., perfumes, lotions, cosmetics), as solvents and plasticizers for cellulose acetate, and in making lacquers, varnishes, and coatings, including those used to provide timed releases in some pharmaceuticals (David *et al.*, 2001; ATSDR, 1995; 2001).

Because phthalates are widely used in many personal care and consumer products, the opportunity is high for non-occupational human exposure. However, to date, the proportional contribution from the various sources and routes of exposure to phthalates is unknown. Traditionally, ingestion has been considered an important route of exposure. Although phthalates have low volatility, they off-gas and are present in residential indoor air (Adibi *et al.*, 2003; Rudel *et al.*, 2003). Dermal contact (ATSDR, 1995; 1997; 2001; 2002) and parenteral exposure from medical devices containing phthalates may also contribute to exposure (ATSDR, 2002). Upon exposure, phthalates are rapidly metabolized and excreted in urine and feces (ATSDR, 1995; 1997; 2001; 2002). The most common biomonitoring approach for investigating human exposure to phthalates is the measurement of urinary concentrations of phthalate metabolites.

In the United States, the National Health Nutrition and Examination Survey (NHANES) is an ongoing survey, conducted by the National Center for Health Statistics at the Centers for Disease Control and Prevention (CDC), designed to collect data on the health and nutritional status of the civilian, noninstitutionalized U.S. population. The data estimates from NHANES, presented by age group, gender, and race/ethnicity, are probability-based, and hence, are representative of the US population. The recent third report from CDC (2005) on the NHANES survey confirmed that human exposure to selected phthalates (i.e., MEP, MBP, MBzP, and MEHP) is widespread among the US population.

As compared to the laboratory animal data on the reproductive toxicity of phthalates, the human data is very limited. All human studies to date were cross-sectional in design, adult exposure levels were measured and relationships with semen parameters were explored. In an early study, Murature *et al.* (1987) recruited 21 university students to explore the relationship between sperm concentration and DBP concentrations in the cellular fractions of ejaculates. The statistical analyses performed were not traditional; that is, they did not treat the subjects as a single population. Instead, the authors assumed that there were two populations that differed in their ability to metabolize DBP. It is not entirely clear, but it seems that the two populations were defined by a visual inspection of DBP concentrations. Based on DBP concentrations, the subpopulations were defined as those with a lower ability to metabolize and those with a greater ability to metabolize DBP. In the subpopulation with a lower ability to metabolize DBP, there was an inverse relationship between sperm concentration and DBP ($r = -0.4$; slope of regression was -0.7). In the subpopulation with a greater ability to metabolize DBP, there was also an inverse correlation of -0.4 (slope of regression -0.6) between DBP and sperm concentration. The study was small and did not measure or adjust for potential confounders.

In India, Rozati *et al.* (2002) studied 21 infertile men with poor semen quality and 32 'control' men with normal semen parameters. Phthalate esters were measured in seminal plasma and the results were reported as the sum of a mixture of DMP, DEP, DBP, BBzP, DEHP, and DnOP. The concentration of phthalates was inversely correlated with sperm morphology ($r = -0.77$, p-value <0.001) and positively correlated with the percentage of single-stranded DNA in sperm ($r = 0.86$, p-value <0.001) assessed with the sperm nuclear chromatin condensation test. The concentration of phthalates was not correlated with ejaculate volume, sperm concentration, or motility. The authors measured total phthalate diesters and did not report results for individual phthalates. The results are noteworthy because they demonstrate the presence of phthalates in seminal plasma. However, because diesters were measured, sample contamination is a potential concern.

Duty *et al.* (2003a; 2003b; 2004) have published three manuscripts exploring the relationships between environmental exposure to phthalates and semen characteristics and sperm DNA damage. Study subjects consisted of male partners of subfertile couples that presented to an infertility clinic in Massachusetts, USA. At the time of the clinic visit, one sample of semen, blood and urine were collected from each subject. Computer-aided sperm analysis (CASA) was used to measure sperm concentration and motility, as well as motion parameters. Strict criteria were used to assess sperm morphology. Sperm DNA damage was assessed with the neutral comet assay. Because the Duty *et al.* (2004) study was ongoing, the number of subjects in each publication varies; however, there is overlap of subjects among the publications.

Among 168 men, they found dose-response relationships (after adjusting for age, abstinence time, and smoking status) between MBP and sperm motility (OR per tertile: 1.0, 1.8, 3.0; p for trend = 0.02) and sperm concentration (OR per tertile: 1.0, 1.4, 5.5; p for trend =0.07) (Duty *et al.*, 2003a). They also found a dose-response relationship between MBzP and sperm concentration (OR per tertile: 1.0, 1.4, 5.5; p for trend = 0.02). There was weak evidence of an association between MBP and sperm morphology, MBzP and sperm motility, and MMP and sperm morphology. Among 220 men, MBP, MBzP and MEHP had inverse associations, although not significant, with VSL (straight line velocity), VCL (curvilinear velocity) and LIN (linearity=VSL/VCL x 100) measured by CASA (Duty *et al.*, 2004). Unexpectedly, positive relationships were found between MEP and both VSL and VCL.

To quantify sperm DNA damage in samples analyzed using the neutral comet assay, Duty *et al.* (2003b) used VisComet image analysis software to measure comet extent, a measure of total comet length (micrometers), percent DNA in tail (tail %), a measure of the proportion of total DNA present in the comet tail, and tail distributed moment (TDM), an integrated measure of length and intensity (micrometers). In multiple regression models, after adjusting for age and smoking status, for an interquartile range (IQR) increase in MEP concentration the comet extent increased by 3.6 μm (95% CI: 0.74, 6.47) and TDM increased by 1.2 μm, (95% CI: -0.05, 2.38). There were no relationships between MBP, MBzP, MEHP and MMP and any comet assay parameters.

In a recently published study from Sweden, Jonsson *et al.* (2005) recruited 234 young Swedish men at the time of their medical conscript examination. Each man provided a single urine sample used to measure concentrations of MEP, MEHP, MBzP, MBP and phthalic acid. Semen quality was assessed using traditional semen parameters and sperm DNA integrity was measured by the sperm chromatin structure assay. Urinary phthalate levels were divided into quartiles and were used to calculate the mean difference and 95 per cent confidence interval between the lowest and highest quartiles. For MEHP, because 63 per cent had urinary concentrations below the detection limit (15 ng/mL), they compared these men with the 18 per cent of men who had the highest concentrations of MEHP. Because multivariate adjusted and unadjusted results differed by less than 15 per cent, potential confounders, such as abstinence time and smoking status, were not kept in the models.

In contrast to the US study, there were no relationships of MBP or MBzP with any of the reproductive markers. MEHP was also not associated with any of the reproductive markers. Men in the highest quartile for MEP had fewer motile sperm (mean difference was 8.8%; 95% confidence interval 0.8, 17) and more immotile sperm (8.9%; 0.3, 18) than men in the lowest MEP quartile. Contrary to their hypothesis, phthalic acid was associated with improved function as measured by more motile sperm and fewer immotile sperm. Phthalic acid is a non-specific marker of phthalate exposure, formed as the result of the hydrolysis of any of the phthalates measured. Interactions between urinary phthalate levels and PCB 153 (measured previously in serum samples from these men) were assessed by including an interaction term in the models. There was no evidence of multiplicative interactions between PCB 153 and any of the phthalates with the reproductive markers (data was not shown). This is in contrast to a previous study by Hauser *et al.* (2005), where they found interactions of MBP and MBzP with PCB 153 in relation to sperm motility.

Although the Swedish study had similarities in design and execution to the US study, there were important differences. The study population in the Swedish study consisted of young men (median age 18 years, range 18-21 years) that were undergoing a medical examination before military service. Since approximately 95 per cent of young men in Sweden undergo the conscript examination, these young men reflected the general population of young Swedish males. In contrast, in the US study, the median age of the men recruited from an infertility clinic was 35.5 years and ranged from 22 to 54 years. None of the men from the infertility clinic were 21 years of age or younger. The recruitment of men from an infertility clinic as compared to young men from the general population may account for some of the differences in results between studies. For instance, it is unclear whether men presenting to an infertility clinic are more 'susceptible' to reproductive toxicants, including phthalates, than men from the general population. Furthermore, it is also unclear whether middle-aged men, as compared to young men, are more susceptible to reproductive toxicants because of an age related response to the toxicant.

Although only 14 per cent of the young Swedish men, as compared to 65 per cent of men in the present study, agreed to participate, it is unlikely that the young Swedish men did so differentially in relation to reproductive function and phthalate levels. Therefore, selection bias as a result of the low participation rate is unlikely in the Swedish study.

Despite similarities in urinary concentrations of the phthalate monoesters across studies, the analytical methods differed between the Swedish and US study. The detection limits for MEP, MBP, MBzP, and MEHP in the Swedish study were 30, 15, 7, 15 ng/mL, many fold higher than the detection limits (~ 1 ng/mL) in the US study. In addition, the precision from comparisons of duplicate analysis on different days was low in the Swedish study and likely due to the lack of isotope-labelled standards for the phthalate monoesters measured. In the US study, the phthalate monoesters were measured using isotope-dilution high performance liquid chromatography tandem mass spectrometry (Blount *et al.*, 2000; Silva *et al.*, 2003; 2004). The isotope dilution method is precise, with relative standard deviations of less than 15 per cent from replicate measurements. The higher limits of detection and lower analytical precision in the Swedish study may contribute to measurement error of urinary phthalate levels and may result in bias to the null hypothesis. However, by categorizing the phthalate levels into quartiles for the statistical analysis, some of the measurement error resulting from the analytical imprecision and low detection limits may be minimized. The Swedish study used urinary creatinine to adjust for urine dilution as compared to specific gravity in the US study. Based on the medians in the tables from the Swedish study, the creatinine adjusts values were quite different from the unadjusted values. In contrast, in the US study, medians between SG-adjusted and unadjusted values were not markedly different.

The statistical methods used for the data analysis also differed between studies and may partially account for the differences in results. In the US study, multivariate logistic regression with categorized semen parameters was used as the primary outcome. Men with all three semen parameters above the reference range were used as comparison subjects in these analyses. In contrast, in the Swedish study, for the primary analysis, semen parameters were used as a continuous measurement and mean differences between men in the highest and lowest phthalate quartiles were calculated. In addition, logistic regression analyses were performed, although the results of these analyses, reported to be consistent with their primary analyses, were not shown. However, it is unclear whether the comparison group in the logistic regression analyses included only men with all three semen parameters above the reference range. If not, dilution of associations between phthalates and semen parameters may occur since the comparison group does not consist of a homogenous group of men with normal semen parameters. For instance, dilution of the associations between sperm concentration and phthalate monoesters may occur if phthalates alter both sperm concentration and motility.

In conclusion, the epidemiologic data on the relationship between semen quality and phthalate exposure remains limited and inconsistent. Although the two large recent studies by Duty *et al. (2004)* and Jonsson *et al. (2005)* had many similarities, important differences existed. The US study recruited older men from an infertility clinic while the Swedish study recruited young men from the general population. It is currently unclear whether these differences in age and recruitment source may partially account for the inconsistent results across studies, especially for MBP and MBzP. Additional studies are critically needed to help elucidate possible explanations for differences across studies, and most importantly to address whether phthalate exposure alter semen quality.

Table 2. Summary table of epidemiologic studies (in chronological order) on the relationship between phthalates and semen quality

Author, country	Study population	Exposure	Results	Comments
Murature *et al.*, 1987 US	21 young men	DBP in cellular fractions of ejaculates	In men with 'low ability to metabolize DBP', inverse relationship between sperm concentration and DBP (r=-0.4; slope of regression was -0.7). In the 'men with a greater ability to metabolize DBP', there was also an inverse correlation of -0.4 (slope of regression -0.6) between DBP and sperm concentration	Small sample size, no adjustment for confounders
Rozati *et al.*, 2002 India	53 men (21 infertile and 32 controls)	Seminal plasma levels of phthalates (DBP, BBzP, DEHP, DnOP)	Sum of phthalates was inversely correlated with sperm morphology (r=-0.77, p-value < 0.001) and positively correlated with the percentage of single-stranded DNA in sperm (r=0.86, p-value <0.001) assessed with the sperm nuclear chromatin condensation test. The concentration of phthalates was not correlated with ejaculate volume, sperm concentration, or motility.	Measured total phthalate diesters, concern with contamination
Duty *et al.*, 2003a, 2004 US	168 men from an infertility clinic (semen parameter), 220 men (CASA results)	Urinary levels of phthalate metabolites (MBP, MBzP, MEP, MEHP, MMP)	Dose-response relationships (after adjusting for age, abstinence time, and smoking status) between MBP and sperm motility (OR per tertile: 1.0, 1.8, 3.0; p for trend = 0.02) and sperm concentration (OR per tertile: 1.0, 1.4, 5.5; p for trend =0.07). Dose-response relationship between MBzP and sperm concentration (OR per tertile: 1.0, 1.4, 5.5; p for trend = 0.02). MBP, MBzP and MEHP had inverse non-significant associations with VSL (straight line velocity), VCL (curvilinear velocity) and LIN (linearity=VSL/VCL x 100).	Confounders considered: age, BMI, abstinence time, smoking status, race

Table 2. continued

Author, country	Study population	Exposure	Results	Comments
Duty *et al.*, 2003b US	168 men from an infertility clinic	Urinary levels of phthalate metabolites (MBP, MBzP, MEP, MEHP, MMP)	After adjusting for age and smoking status, for an interquartile range increase in MEP concentration the comet extent increased by 3.6 μm (95% CI: 0.74, 6.47) and tail distributed moment increased by 1.2 μm, (95% CI: -0.05, 2.38). There were no relationships between MBP, MBzP, MEHP and MMP and any comet assay parameters.	Confounders considered: age, BMI, abstinence time, smoking status, race
Jonsson *et al.*, 2005 Sweden	234 young men	Urinary levels of MEP, MEHP, MBzP, MBP and phthalic acid	No relationships of MBP, MBzP, or MEHP with any of the semen parameters. The highest quartile for MEP had fewer motile sperm (mean difference was 8.8%, 95% CI: 0.8, 17) and more immotile sperm (8.9%, 95% CI: 0.3, 18). Phthalic acid was associated with improved function as measured by more motile sperm and fewer immotile sperm.	Confounders considered: abstinence time, smoking status.

Abbreviations: di(2-ethylhexyl) phthalate [DEHP]; monoethylhexyl phthalate [MEHP]; di-isononyl phthalate [DiNP]; di-n-octyl phthalate [DnOP]); diethyl phthalate [DEP]; monoethyl phthalate [MEP]; butylbenzyl phthalate [BBzP]; dibutyl phthalate [DBP]; monobutyl phthalate [MBP]; monobenzyl phthalate [MBzP].

5. Polychlorinated biphenyls and dichlorodiphenyl trichloroethane (DDT)

Polychlorinated biphenyls (PCBs) are a class of synthetic, persistent, lipophilic, halogenated aromatic compounds that were widely used in industrial and consumer products for decades before their production was banned in the late 1970's. PCBs were used in cutting oils, lubricants and as electrical insulators. As a result of their extensive use and persistence, PCBs remain ubiquitous environmental contaminants. They are distributed worldwide and have been measured in air, water, aquatic and marine sediments, fish, and wildlife (De Voogt and Brinkman, 1989). Furthermore, they are biologically concentrated and stored in human adipose tissue. The general population is exposed primarily through ingestion of contaminated foods (e.g., fish, meat, and dairy products), as PCBs can bioaccumulate up the food chain. However, exposure may also occur through dermal contact (soil and house dust) and inhalation (indoor air in residential buildings and workplaces, as well as outdoor air). For example, in the 1960s and 1970s, PCBs were used in sealants for commercial building construction, and high levels of PCBs (up to 36,000 ppm) have been found to remain in the caulking of some public buildings that may lead to contamination of indoor air and dust (Herrick *et al.*, 2004). As a result of their persistence and ubiquity, measurable levels of serum PCBs are found in the majority of the U.S. general population (Longnecker *et al.*, 1997). Serum levels of PCBs are an integrated measure of internal dose, reflecting exposure from all sources over the previous years; depending on the congener, the half-life of PCBs in the blood ranges from one to ten or more years (Brown, 1994; Phillips *et al.*, 1989).

5.1. Studies on environmental exposure

In an early study on PCBs and semen quality, Bush *et al.* (1986) studied semen samples from fertile men (n = 33), men with oligospermia (n = 50) or azoospermia (n = 50) and men status post-vasectomy (n = 25). The average (SD) age of the men from these groups was 33 (7), 32 (4), 33 (5), 38 (7) years, respectively. The seminal concentrations of PCBs 153, 138, and 118 were inversely related to sperm motility only among samples with a sperm count less than 20 million/mL. The authors caution against over interpreting these associations because they were found only among a subset of subjects.

In the Netherlands, Dallinga *et al.* (2002) studied the relationship between PCBs and semen quality among men that were partners in couples visiting an infertility treatment center. They identified two groups of men, those men with good semen quality (n = 31) and men with very poor semen quality (n = 34) based on three semen samples. Progressive motile sperm concentration was used to make the classification. A Makler counting chamber was used to measure sperm concentration and motility and strict criteria were used for sperm morphology. Blood and semen were analyzed for PCB 118, 138, 153, and 180 and their hydroxylated metabolites. The mean (SD) non-lipid adjusted levels of PCB 153 were 0.41 (0.22) ng/g blood. Assuming that serum percent lipid is approximately 0.5 per cent, the estimated lipid adjusted concentration would be 82 ng/g lipids. Contrary to expectations, the sum of PCBs in seminal plasma of men with good semen quality was higher than among men with poor semen quality (0.071 ng/mL and 0.022 ng/mL seminal plasma, respectively, p-value = 0.06). However, within the group of men with good semen quality, there were inverse associations between serum levels of sum of PCB metabolites and sperm count (p-value = 0.04) and progressive motile sperm concentration (p-value = 0.02). There were also negative non-significant corresponding associations in the men with poor semen quality. Because associations with semen quality were found for PCB metabolites and not the parent PCBs, these results suggested that the PCB metabolites were the biologically active compounds.

Richthoff *et al.* (2003) conducted a study on the relationship between PCB 153 and semen parameters among 305 young men undergoing a conscript examination for military service. The men ranged in age from 18 to 21 years with a median age of 18.1 years. PCB 153 levels were considered representative of background environmental levels for men from Southern Sweden; the median was 65 ng/g lipid with a range from 23 to 250 ng/g lipid. PCB 153 is a good biomarker of exposure to total PCBs and toxic equivalents (TEQ) (Gladen *et al.*, 1999; Grimvall *et al.*, 1997). Semen samples were analyzed according to WHO recommendations (1999). Sperm concentration was assessed by a modified Neubauer chamber. Sperm motility (categories A, B, C, and D) was assessed according to WHO recommendations and the percentage of motile sperm was assessed by use of CRISMAS computer-aided sperm motility analyzer (CASA) with a Makler chamber. The following confounders were considered for inclusion in the models: BMI, abstinence period, and smoking status. There were significant inverse associations between PCB 153 and percent motile sperm; a 10ng/g lipid increase in PCB 153 was associated with a 1.0 per cent decline in percent CASA motile sperm (95% CI: -2.0, -0.13). The association between PCB 153 and conventional sperm motility was slightly weaker. There were no associations between PCB 153 and sperm concentration or total sperm count. The study was relatively large and well conducted. Although the participation rate was very low, only 13.5 per cent of eligible subjects agreed to participate, it is unlikely that this would introduce bias since young men are likely to be unaware of their fertility or exposure levels.

Rozati *et al.* (2002) measured PCBs in seminal plasma and explored relationships with semen parameters among men in India. Details of the study are provided above in the phthalate section. PCBs were detected in the seminal plasma of infertile men but not controls. They reported a negative correlation between seminal plasma PCB levels and total progressive motility (r = -0.5) and a positive correlation with percentage of single-stranded DNA in sperm (r = 0.6). No correlations were found between PCBs and sperm count, rapid progressive motility or normal morphology. The authors reported results for total PCBs and not for individual congeners.

Potential confounders were considered in the method section but no adjustments were made.

Rignell-Hydbom *et al.* (2005; 2004) reported on the associations between PCBs and p,p'-DDE with semen parameters and sperm chromatin integrity. Swedish fishermen from the east and west coasts were studied. 195 Swedish fishermen (median age 50.6 years, ranged from 24-65 years) participated in the semen quality study and 176 of these men had semen samples analyzed for sperm chromatin integrity, assessed by sperm chromatin structure assay (SCSA). The median serum levels of PCB 153 and p,p'-DDE (dichlorodiphenyl-dichloroethene) were 193 ng/g lipid (ranged from 39 to 1,460) and 240 ng/g lipid (ranged from 334 to 2,251), respectively. When PCB 153 was categorized into quintiles, the highest quintile had decreased sperm motility compared with men in the lowest quintile. The age adjusted mean difference was 9.9 per cent (95% confidence interval -1.0 to 21%, p-value = 0.08). There were no consistent associations of PCB 153 with sperm concentration. Although p,p'-DDE was inversely associated with sperm motility, when age was included in the models the association became weaker and non-significant. Among men with SCSA results, there was a univariate association between log-transformed PCB 153 and the percentage of sperm showing DNA fragmentation (%DFI), $r = 0.27$, p-value < 0.001). When age was adjusted for the association was no longer significant (p-value = 0.28). However, in age adjusted analyses in which PCB 153 was divided into quintiles, the lowest quintile had significantly lower %DFI (p-values < 0.006). Although PCB 153 and p,p'-DDE were highly correlated ($r = 0.8$), the relationship between p,p'-DDE and %DFI was less consistent (p-value = 0.1). Interestingly, there was a moderate correlation between sperm motility and %DFI, which suggests that PCB 153 may alter both through a common pathway or that alteration of one may in turn affect the other parameter.

Hauser and colleagues (2003a) conducted a study on 212 male partners of sub-fertile couples visiting an infertility clinic in Massachusetts, US. Sperm concentration and motility were assessed with CASA and morphology with the strict criteria. The mean (SD) age was 36.0 (5.4) years. 57 PCB congeners were measured, and included PCB 118, 138, 153 and 180. The median levels for PCB 153 and p,p'-DDE were 42 ng/g lipid (range 9.3 to 361) and 222 ng/g lipid (range 64 to 8912), respectively. Multivariate logistic regression analyses were used in which semen parameters were dichotomized based on WHO reference values (1999). The comparison groups for each analysis were defined as men with all three semen parameters above reference values. There were significant dose-response relationships (odds ratio per tertile adjusted for age, abstinence time, and smoking status) between PCB 138 and below reference sperm motility (1.00, 1.68, 2.35, respectively; p-value for trend 0.04) and sperm morphology (1.00, 1.36, 2.53; p-value=0.04). Associations between semen parameters and PCB 153 were not consistent. DDE showed a weak non-significant relationship with sperm motility. Hauser *et al.* (2003b) also studied the relationship between PCBs and p,p'-DDE with DNA integrity in sperm using the neutral comet assay. They did not find any strong or consistent associations between any of the PCB congeners and p,p'-DDE with measures of DNA integrity.

Although the pesticide DDT was banned for use in most industrialized countries, it is currently used for malaria control in several countries. Ayotte *et al.* (2001) reported on the association between p,p'-DDE, a major biologically persistent metabolite of DDT, and semen quality in 24 young men from Chiapas, Mexico. The men, 16 to 28 years of age, were non-occupationally exposed to DDT. The mean concentration of p,p'-DDE was 77.9 mg/kg (range, 17 to 177), a value several hundred fold higher than levels in men from other countries, such as the US and Canada, where DDT was not recently used. p,p'-DDE was inversely correlated with both semen volume ($r = -0.47$) and sperm count ($r = 0.4$). Although the study was small and did not control for potential confounders, the results are intriguing and worthy of replication in other cohorts.

5.2. High PCB exposure studies

Guo *et al.* (2000) studied the relationship between semen quality and prenatal exposure to PCBs and PCDFs after the poisoning episode in Taiwan in 1979 in which PCB contaminated rice oil was ingested. In 1998, 12 men pre-natally exposed to contaminated rice oil and 23 healthy unexposed subjects of comparable age provided a semen sample. The unexposed men had no unusual chemical exposure and were recruited from a local high school. Sperm motility was assessed using a Makler chamber and also by CASA. Morphology was assessed using the WHO guidelines. The mean (SD) age of the exposed men was 17.3 (1.2) years and 17.6 (1.0) for the unexposed men. The proportion of sperm with abnormal morphology was increased in the exposed men (37.5% as compared to 25.9% for unexposed men). In the exposed men the percentage of motile sperm (35.1% compared to 57.1% in unexposed men) and rapidly motile sperm (25.5% compared to 42.4 % in unexposed men) were reduced. Several of the CASA parameters were reduced in the exposed men, in particular, average path velocity (VAP), straight-line velocity (VSL), and curvilinear velocity (VCL). Sperm from exposed men had reduced hamster oocyte penetration as compared to unexposed men. This small study provided the opportunity to explore high pre-natal exposure to PCBs and PCDFs.

In another study on men from the Taiwan PCB poisoning, Hsu *et al.* (2003) studied the relationship between semen quality and levels of PCBs among men that consumed contaminated rice oil some twenty years earlier. They identified 40 exposed men and 28 unexposed men that were matched using an address registry. Mean age of exposed (37.9 years) and unexposed (40.4) were similar. Exposed men had a higher percentage of sperm with abnormal morphology (27.5% compared to 23.3%) and a higher oligospermia rate (9% compared to 1%). The ability of sperm to penetrate the hamster oocyte was reduced in exposed men. The results of this small study provide evidence of adverse effects of exposure to PCBs and PCDFs among men exposed 20 years earlier to the contaminated rice oil.

Table 3. Summary table of epidemiologic studies (in chronological order) on the relationship of polychlorinated biphenyls and p,p-DDE with semen quality

Author	Study population	Exposure	Results	Comments
Bush *et al.*, 1986	33 fertile, 50 subfertile, 50 infertile, and 25 post-vasectomy men	Seminal plasma levels of PCBs and p,p'-DDE	PCB 153, 138, and 118 were inversely related to sperm motility only among samples with a sperm count less than 20 million/mL. No associations of semen parameters with p,p'-DDE.	Association found only among a subset of men.
Rozati *et al.*, 2002	53 men from India (21 infertile and 32 controls)	Seminal plasma levels of PCBs	PCBs detected in the seminal plasma of infertile men but not controls. Negative correlation between PCBs and total progressive motility (r=-0.5), and positive correlation with percentage of single-stranded DNA in sperm (r=0.6). No correlations with sperm count, rapid progressive motility or normal morphology.	Data on individual PCB congeners not presented. No statistical adjustment for potential confounders.

Table 3. continued.

Author	Study population	Exposure	Results	Comments
Dallinga *et al.*, 2002	65 Dutch men from an infertility clinic	Serum and semen levels of PCB 118, 138, 153, 180 and their metabolites	Seminal plasma PCB levels among men with good semen quality were higher than among men with poor semen quality (p-value =0.06). In men with good semen quality, there were inverse associations between serum levels of sum of PCB metabolites and sperm count (p-value = 0.04) and progressive motile sperm concentration (p-value = 0.02). There were also negative non-significant corresponding associations in men with poor semen quality.	Confounders considered: age and smoking status. Measured PCB metabolites.
Richthoff *et al.*, 2003	305 Swedish young men	Serum levels of PCB 153	Inverse association between PCB 153 and percent motile sperm (10 ng/g lipid increase in PCB153 associated with a 1.0% decline in percent CASA motile sperm (95% CI: -2.0, -0.13)). No association of PCB 153 with sperm concentration.	Confounders considered: BMI, abstinence period, smoking status.
Hauser *et al.*, 2003	212 US men from an infertility clinic	Serum levels of PCBs and p,p'-DDE	Dose-response relationships (odds ratio per tertile adjusted for age, abstinence time, and smoking status) between PCB 138 and below reference sperm motility (1.00, 1.68, 2.35, respectively; p-value for trend 0.04) and sperm morphology (1.00, 1.36, 2.53; p-value=0.04). DDE had a non-significant association with sperm motility.	Confounders considered: BMI, age, abstinence period, smoking status.

Table 3. continued.

Author	Study population	Exposure	Results	Comments
Hauser *et al.*, 2003	212 US men from an infertility clinic	Serum levels of PCBs and p,p'-DDE	No associations between any of the comet assay parameters and PCBs or DDE.	Confounders considered: age, smoking status.
Rignell-Hydbom *et al.*, 2004	195 Swedish fishermen	Serum levels of PCB 153 and p,p'-DDE	The highest PCB 153 quintile had decreased sperm motility as compared with men in the lowest quintile. The age adjusted mean difference was 9.9% (95% confidence interval -1.0 to 21%, p-value=0.08). No significant associations of p,p-DDE with semen parameters.	Confounders considered: age, smoking status, abstinence time, BMI, reproductive hormones.
Rignell-Hydbom *et al.*, 2005	176 Swedish fishermen	Serum levels of PCB 153 and p,p'-DDE	Age adjusted analyses, the lowest PCB quintile had significantly lower %DFI than the other quintiles (p-values < 0.006). Non-significant relationship between p,p'-DDE and %DFI (p-value = 0.1).	Confounders considered: age, smoking status, abstinence time, BMI, reproductive hormones.
High Exposure Studies				
Guo *et al.*, 2000	35 young men from Taiwan (12 pre-natally exposed to contaminated rice oil, 23 unexposed men)	Maternal ingestion (yes/no) of rice oil contaminated with PCBs and PCDFs	Increased abnormal morphology in exposed men (37.5%) as compared to unexposed men (25.9%) for unexposed men. Exposed men had decreased percentage of motile sperm (35.1% compared to 57.1% in unexposed men) and rapidly motile sperm (25.5% compared to 42.4 % in unexposed men). Reduced hamster oocyte penetration in exposed men.	Age and % smokers in exposed and unexposed groups were similar. No statistical adjustment for confounders.

Table 3. continued.

Author	Study population	Exposure	Results	Comments
Hsu *et al.*, 2003	68 men from Taiwan (40 exposed to contaminated rice oil and 28 unexposed)	Ingestion (yes/no) of rice oil contaminated with PCBs and PCDFs	Exposed men had higher percentage of sperm with abnormal morphology (27.5%) compared to unexposed men (23.3%), and a higher oligospermia rate (9% compared to 1%, respectively). Ability of sperm to penetrate the hamster oocyte was reduced in exposed men.	Age and % smokers in exposed and unexposed groups were similar. No statistical adjustment for confounders

Abbreviations: body mass index [BMI], polychlorinated biphenyls [PCBs], polychlorinated dibenzofurans [PCDFs]

The data on the relationship between PCBs and semen quality support an inverse association of PCBs with reduced semen quality, specifically reduced sperm motility. The associations found were generally consistent across studies performed in different countries (India, the Netherlands, Taiwan, Sweden, and US) that used different methods to measure semen quality and PCBs. Furthermore, associations were consistently found despite a range of PCB levels, that is there did not appear to be a threshold. The PCB levels in these studies ranged from low background levels (Hauser *et al.,* 2003a; 2003b; Richthoff *et al.* 2003; Dallinga *et al.*, 2002), to high background levels due to consumption of contaminated fish (Rignell-Hydbom *et al.,* 2004; 2005a; 2005b), to even higher exposure levels due to ingestion of contaminated rice oil (Guo *et al.,* 2000; Hsu *et al.*, 2003). Although the data across studies generally support a relationship between PCBs and poor semen quality, there are possible alternative explanations. One potential alternative explanation is that PCBs are a surrogate for exposure to other environmental factors that may predict semen quality. Although this is possible, there is currently no evidence identifying potential alternative exposures. Another explanation is that there may be confounding of the associations by some currently unrecognized or unmeasured confounders. Although possible, this is also unlikely because the more recent studies considered important potential confounders and the results were consistent across studies suggesting that it is unlikely that there is a strong unmeasured confounder. In conclusion, although PCBs are no longer used, this data, along with ongoing human exposure, albeit at lower levels than several decades ago, raise concerns regarding altered human fertility due to adverse affects on semen quality.

6. Emerging compounds

In addition to the chemicals discussed in this chapter, there are other classes of chemicals that require further study as to their relation with human semen quality. These chemicals include alkylphenols, such as 4-nonylphenol, bisphenol A (BPA) and fluorinated organic compounds such as perfluorooctane octanoate (PFOA) and perfluorooctane sulfonate (PFOS). Alkylphenols are used as surface active agents in cleaning/washing agents, paints, and cosmetics, while BPA is used in the manufacture of polycarbonate plastics and epoxy resins. The perfluorinated compounds are used to make fabrics stain-resistant/water repellant and in coatings on cookware and other products. Although human exposure to these chemicals has been demonstrated, the health effects data in humans remains severely limited.

7. Conclusions

This chapter presents an up-to-date summary of recently published human studies on the relationship between semen quality and exposure to several classes of environmental chemicals. The classes of chemicals included in this chapter represent a number of contemporary and widely used compounds such as insecticides and phthalates. In addition, although PCBs were banned several decades ago, they are also discussed because over the last five years many well-designed epidemiologic studies have been published.

Recent studies, along with several earlier studies, suggested that exposure to specific non-persistent insecticides may be associated with poorer semen quality. Two occupational studies of workers exposed to the insecticide carbaryl reported suggestive evidence of lowered sperm concentration and morphology, while a more recent study of environmental exposure to carbaryl found an association with reduced sperm concentration and motility. Recent evidence also suggested a relationship between occupational and environmental exposure to some organophosphorus insecticides and reduced sperm concentration and motility.

Solvents, like pesticides, represent a large number of chemicals with varying reproductive toxicities. Adverse effects on semen quality associated with exposure to some solvents, such as ethylene glycol ethers, were recognized, while potential adverse effects on the human reproductive system for many other solvents were not well-studied. As new chemicals are introduced and more studies are conducted, evidence of effects on semen quality in relation to solvent exposure may emerge as in the recent case of 2-bromopropane.

For phthalates, a widely used class of chemicals, there was suggestive but inconsistent evidence for an inverse relationship with semen quality, specifically between monobutyl and monobenzyl phthalate and sperm motility and concentration. For exposure to PCBs, there was consistent evidence of associations with poorer sperm motility. The inverse associations between PCBs and sperm motility were consistent across studies conducted in several different countries (India, the Netherlands, Taiwan, Sweden, and US) despite the use of different methods to measure semen quality and PCBs. Furthermore, the inverse associations were also consistently found despite a range of PCB levels, that is there did not appear to be a threshold.

Although the epidemiologic data on these historic, contemporary and emerging environmental contaminants suggest that there may be associations with altered semen quality, the quantity and quality of the data available for the different types of compounds varied. For example, though there are hundreds of different pesticides currently in use worldwide, limited human data existed on male reproductive endpoints for only a select few. Also, for some of these chemicals, such as phthalates, the data across studies was not entirely consistent. For instance, one study found associations of semen quality with monobutyl phthalate and monobenzyl phthalate while another large epidemiologic study did not. The limited human data, and in certain instances inconsistent data across studies, highlight the need for further epidemiological research on these classes of chemicals.

A future challenge to understanding the relationship between these chemicals and semen quality includes the changes in exposure levels among populations over time due to the ever-changing patterns of production and use of these compounds. Another challenge is to understand how simultaneous coexposures to these chemicals may affect semen quality. It is well known that humans are exposed to all of these compounds simultaneously, as well as to many other chemicals. However, there is limited data on the interactions between chemicals within a class or across classes of chemicals. Chemicals may interact additively or multiplicatively, or antagonistically. The human health risks of exposure to chemical mixtures is very understudied. Despite these challenges, evolving and innovative technologies designed to improve the assessment of human exposure and male reproductive health endpoints should provide enhanced opportunities for improving our understanding of the relationship between environmental chemicals and semen quality. Innovations include improved biomarkers of exposure and more sophisticated statistical methods that deal with multiple exposures simultaneously.

References

Abell, A., Ernst, E., and Bonde, J.P. (2000) Semen quality and sexual hormones in greenhouse workers, *Scand. J. Work Environ. Health* **26**, 492-500.

Adami, H.O., Bergstrom, R., Mohner, M., Zaonski, W., Storm, H., Ekbom, A., Tretli, S., Teppo, L., Ziegler, H., Rahu, M., Gurevicius, G., and Stengrevics, A. (1994) Testicular cancer in nine northern European countries, *Int. J. Cancer* **59**, 33-38.

Adibi, J.J., Perera, F.P., Jedrychowski, W., Camann, D.E., Barr, D., Jacek, R., and Whyatt, R.M. (2003) Prenatal exposures to phthalates among women in New York City and Krakow, Poland, *Environ. Health Perspect.* **111**, 1719-1722.

Anway, D., Cupp, A.S., Uzumcu, M., and Skinner, M.K. (2005) Epigenetic transgenerational actions of endocrine disrupters and male fertility, *Science* **308**, 1466-1469.

ATSDR - Agency for Toxic Substances and Disease Registry (2002) Toxicological profile for di(2-ethylhexyl)phthalate (DEHP), Atlanta, GA.

ATSDR - Agency for Toxic Substances and Disease Registry (1995) Toxicological profile for diethyl phthalate (DEP), Atlanta, GA.

ATSDR - Agency for Toxic Substances and Disease Registry (1997) Toxicological profile for di-n-octyl phthalate (DNOP), Atlanta, GA.

ATSDR - Agency for Toxic Substances and Disease Registry (2001) Toxicological profile for di-n-butyl phthalate (DBP), Atlanta, GA.

Ayotte, P., Giroux, S., Dewailly, E., Avila, M.H., Farias, P., Danis, R., and Diaz, C.V. (2001) DDT spraying for malaria control and reproductive function in Mexican men, *Epidemiology* **12**, 366-367.

Bergstrom, R., Adami, H.O., Mohner, M., Zatonski, W., Storm, H., Ekbom, A., Tretli, S., Teppo, L., Akre, O., and Hakulinen, T. (1996) Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon, *JNCI* **88**, 727-733.

Bian, Q., Xu, L.C., Wang, S.L., Xia, Y. K., Tan, L. F., Chen, J. F., Song, L., Chang, H. C., and Wang, X. R. (2004) Study on the relation between occupational fenvalerate exposure and spermatozoa DNA damage of pesticide factory workers, *Occup. Environ. Med.* **61**, 999-1005.

Blount, B.C., Milgram, K.E., Silva, M.J., Malek, N.A., Reidy, J.A., Needham, L.L., and Brock, J.W. (2004) Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS, *Anal. Chem.* **72**, 4127-4134.

Boekelheide, K., Darney, S.P., Daston, G. P., David, R. M., Luderer, U., Olshan, A. F., Sanderson, W. T., Willhite, C. C., and Woskie, S. (2004) NTP-CERHR Expert Panel Report on the reproductive and developmental toxicity of 2-bromopropane, *Reprod. Toxicol.* **18**, 189-217.

Bonde, J.P., and Giwercman, A. (1995) Occupational hazards to male fecundity, *Reprod. Med. Rev.* **4**, 59-73.

Brown, J.F. (1994) Determination of PCB metabolic, excretion, and accumulation rates for use as indicators of biological response and relative risk, *Environ. Sci. Technol.* **28**, 2295-2305.

Burgess, W.A. (1995) *Recognition of Health Hazards in Industry: A Review of Materials and Processes*, Wiley & Sons, New York, 59-62.

Bush, B., Bennett, A.H., and Snow, J.T. (1986) Polychlorobiphenyl congeners, p,p'-DDE, and sperm function in humans, *Arch. Environ. Contam. Toxicol.* **15**, 333-341.

Carlsen, E., Giwercman, A., Keiding, N., and Skakkebaek, N.E. (1992) Evidence for decreasing quality of semen during past 50 years, *Brit. Med. J.* **305**, 609-613.

CDC - Centers for Disease Control and Prevention (2005) *Third National Report on Human Exposure to Environmental Chemicals*, Centers for Disease Control and Prevention; National Center for Environmental Health; Division of Laboratory Sciences: Atlanta, GA, [online] http://www.cdc.gov/exposurereport/3rd/pdf/thirdreport.pdf [10/10/2005].

Cherry, N., Labreche, F., Collins, J., and Tulandi, T. (2001) Occupational exposure to solvents and male infertility, *Occup. Environ. Med.* **58**, 635-640.

Chia, S. E., Ong, C. N., Tsakok, M. F., and Ho, A. (1996) Semen parameters in workers exposed to trichloroethylene, *Reprod. Toxicol.* **10**, 295-299.

Dallinga, J.W., Moonen, E.J.C., Dumoulin, C.M., Evers, J.L.H., Geraedts, J.P.M., and Kleinjans, C.S. (2002) Decreased human semen quality and organochlorine compounds in blood, *Hum. Reprod.* **17**, 1973-1979.

David, R.M., McKee, R.H., Butala, J.H., Barter, R.A., and Kayser, M. (2001) Esters of aromatic mono-, di-, and tricarboxylic acids, aromatic diacids, and di-, tri-, or polyalcohols, In E. Bingham, B. Cohrssen, C.H. Powell (eds.) *Patty's Toxicology*, New York: John Wiley and Sons, 635-932.

De Voogt, P., and Brinkman, U.A.T. (1989) Production properties and usage of polychlorinated biphenyls, in Kimbrough R.D., Jensen A.A., (eds.), *Halogenated Biphenyls, Terphenyls, Naphthalenes, dibenzodioxins and related products, 2nd ed.* Elsevier-North Holland, Amsterdam, 3-45.

Duty, S.M., Calafat, A.M., Silva, M.J., Brock, J.W., Ryan, L., Chen, Z., Overstreet, J., and Hauser, R. (2004) The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters, *J. Androl.* **25**, 293-302.

Duty, S.M., Silva, M.J., Barr, D.B., Brock, J.W., Ryan, L., Chen, Z., Herrick, R.F., Christiani, D.C., and Hauser, R. (2003a) Phthalate exposure and human semen parameters, *Epidemiology* **14**, 269-277.

Duty, S.M., Singh, N.P., Silva, M.J., Barr, D.B., Brock, J.W., Ryan, L., Herrick, R.F., Christiani, D.C., and Hauser, R. (2003b) The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay, *Environ. Health Perspect.* **111**, 1164-1169.

Figa-Talamanca, I., Traina, M.E., and Urbani, E. (2001) Occupational exposures to metals, solvents and pesticides: recent evidence on male reproductive effects and biological markers, *Occup. Med.* **51**, 174-188.

Gladen, B.C., Longnecker, M.P., and Schecter, A.J. (1999) Correlations among polychlorinated biphenyls, dioxins, and furans in humans, *Am. J. Ind. Med.* **35**, 15-20.

Grimvall, E., Rylander, L., Nilsson-Ehle, P., Nilsson, U., Stromberg, U., Hagmar, L., and Ostman, C. (1997) Monitoring of polychlorinated biphenyls in human blood plasma: methodological developments and influence of age, lactation, and fish consumption, *Arch. Environ. Contam. Toxicol.* **32**, 329-336.

Guo, Y.L., Hsu, P.C., Hsu, C.C., and Lambert, G.H. (2000) Semen quality after prenatal exposure to Polychlorinated biphenyls and dibenzofurans, *Lancet* **356**, 1240-1241.

Hauser, R. The Environment and Male Fertility: Recent Research on Emerging Chemicals and Semen Quality, *Seminars Reprod. Med.* (In Press).

Hauser, R., Chen, Z., Pothier, L., Ryan, L., and Atshul, L. (2003a) The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and p,p'-DDE, *Environ. Health Perspect.* **111**, 1505-1511.

Hauser, R., Singh, N.P., Chen, Z., Pothier, L., Ryan, L., and Atshul, L. (2003b) Lack of an association between environmental exposure to polychlorinated biphenyls and p,p'-DDE and DNA damage in human sperm measured using the neutral comet assay, *Hum. Reprod.* **18**, 2525-2533.

Hauser, R., Williams, P., Altshul, L., and Calafat, A. (2005) Evidence of interaction between polychlorinated biphenyls and phthalates in relation to human sperm motility, *Environ. Health Perspect.* **113**, 425-430.

Herrick, R.F., McClean, M.D., Meeker, J.D., Baxter, L.K., and Weymouth, G.A. (2004) An unrecognized source of PCB contamination in schools and other buildings, *Environ. Health Perspect.* **112**, 1051-1053.

Hsu, P.C., Huang, W., Yao, W., Hsing, M.H., Guo, Y.L., Lambert, G.H. (2003) Sperm changes in men exposed to polychlorinated biphenyls and dibenzofurans, *JAMA* **289**, 2943-2944.

Huyghe, E., Matsuda, T., and Thonneau, P. (2003) Increasing incidence of testicular cancer worldwide: a review, *J. Urol.* **17**, 5-11.

Ichihara, G., Asaeda, N., Kumazawa, T., Tagawa, Y., Kamijima, M., Yu, X., Kondo, H., Nakajima, T., Kitoh, J., Yu, I., Moon, Y., Hisanaga, N., and Takeuchi, Y. (1997) Testicular and hematopoietic toxicity of 2-bromopropane, a substitute for ozone layer-depleting chlorofluorocarbons, *J. Occup. Health* **39**, 57-63.

Ichihara, G., Ding, X., Yu, X., Wu, X., Kamijima, M., Peng, S., Jiang, X., and Takeuchi, Y. (1999) Occupational health survey on workers exposed to 2-bromopropane at low concentrations, *Am. J. Ind. Med.* **35**, 523-531.

Jonsson, B.A.G., Richthoff, J., Rylander, L., Giwercman, A., and Hagmar, L. (2005) Urinary phthalate metabolites and biomarkers of reproductive function in young men, *Epidemiology* **16**, 487-493.

Juhler, R.K., Larsen, S.B., Meyer, O., Jensen, N.D., Spano, M., Giwercman, A., and Bonde, J.P. (1999) Human semen quality in relation to dietary pesticide exposure and organic diet, *Arch. Environ. Contam. Toxicol.* **37**, 415-423.

Kamijima, M., Hibi, H., Gotoh, M., Taki, K., Saito, I., Wang, H., Itohara, S., Yamada, T., Ichihara, G., Shibata, E., Nakajima, T., and Takeuchi, Y. (2004) A survey of semen indices in insecticide sprayers, *J. Occup. Health* **46**, 109-118.

Kim, Y., Jung, K., Hwang, T., Jung, G., Kim, H., Park, J., Kim, J., Park, D., Park, S., Choi, K., and Moon, Y. (1996) Hematopoietic and reproductive hazards of Korean electronic workers exposed to solvents containing 2-bromopropane, *Scand. J. Work Environ. Health* **22**, 387-391.

Kolstad, H.A., Bonde, J.P., Spano, M., Giwercman, A., Zschiesche, W., Kaae, D., Larsen, S.B., and Roeleveld, N. (1999) Change in semen quality and sperm chromatin structure following occupational styrene exposure, *Int. Arch. Occup. Environ. Health* **72**, 135-141.

Lahdetie, J. (1995) Occupation- and exposure-related studies on human sperm, *J. Occup. Environ. Med.* **37**, 922-930.

Larsen, S.B., Giwercman, A., Spano, M., and Bonde, J.P. (1998) A longitudinal study of semen quality in pesticide spraying Danish farmers, The ASCLEPIOS Study Group, *Reprod. Toxicol.* **12**, 581-589.

Longnecker, M.P., Rogan, W.J., and Lucier, G. (1997) The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBS (polychlorinated biphenyls) and an overview of organochlorines in public health, *Annu. Rev. Public Health* **18**, 211-244.

Meeker, J.D., Ryan, L., Barr, D.B., Herrick, R.F., Bennett, D.H., Bravo, R., and Hauser, R. (2004a) The relationship of urinary metabolites of carbaryl/naphthalene and chlorpyrifos with human semen quality, *Environ. Health Perspect.* **112**, 1665-1670.

Meeker, J.D., Singh, N.P., Ryan, L., Duty, S.M., Barr, D.B., Herrick, R.F., Bennett, D.H., and Hauser, R. (2004b) Urinary levels of insecticide metabolites and DNA damage in human sperm, *Hum. Reprod.* **19**, 2573-2580.

Murature, D.A., Tang, S.Y., Steinhardt, G., and Dougherty, R.C. (1987) Phthalate-esters and semen quality parameters, *Biomed. Environ. Mass. Spectrom.* **14**, 473-477.

Oliva, A., Spira, A., and Multigner, L. (2001) Contribution of environmental factors to the risk of male infertility, *Hum. Reprod.* **16**, 1768-1776.

Padungtod, C., Hassold, T.J., Millie, E., Ryan, L.M., Savitz, D.A., Christiani, D.C., and Xu, X. (1999) Sperm aneuploidy among Chinese pesticide factory workers: scoring by the FISH method, *Am. J. Ind. Med.* **36**, 230-238.

Padungtod, C., Savitz, D.A., Overstreet, J.W., Christiani, D.C., Ryan, L.M., and Xu, X. (2000) Occupational pesticide exposure and semen quality among Chinese workers, *J. Occup. Environ. Med.* **42**, 982-992.

Paulozzi, L. (1999) International trends in rates of hypospadias and cryptorchidism, *Environ. Health Perspect.* **107**, 297-302.

Pflieger-Bruss, S., Schuppe, H.C., and Schill, W.B. (2004) The male reproductive system and its susceptibility to endocrine disrupting chemicals, *Andrologia* **36**, 337-345.

Phillips, D.L., Smith, A.B., Burse, V.W., Steele, G., Needham, L.L., and Hannon, H.H. (1989) Half-life of polychlorinated biphenyls in occupationally exposed workers, *Arch. Environ. Health.* **44**, 351-354.

Ratcliffe, J.M., Schrader, S.M., Clapp, D.E., Halperin, W.E., Turner, T.W., and Hornung, R.W. (1989) Semen quality in workers exposed to 2-ethoxyethanol, *Br. J. Ind. Med.* **46**, 399-406.

Recio, R., Robbins, W.A., Borja-Aburto, V., Moran-Martinez, J., Froines, J.R., Hernandez, R. M., and Cebrian, M.E. (2001) Organophosphorous pesticide exposure increases the frequency of sperm sex null aneuploidy, *Environ. Health Perspect.* **109**, 1237-1240.

Richthoff, J., Rylander, L., Jonsson, B.A.G., Akesson, H., Hagmar, L., Nisson-Ehle, P., Stridsberg, M., and Giwercman, A. (2003) Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population, *Environ. Health Perspect.* **111**, 409-413.

Rignell-Hydbom, A., Rylander, L., Elzanaty, S., Giwercman, A., Lindh, C.H., Eleuteri, P., Rescia, M., Leter, G., Cordelli, E., Spano, M., and Hagmar, L. (2005) Exposure to PCBs and p,p'-DDE and human sperm chromatin integrity, *Environ. Health Perspect.* **113**, 175-179.

Rignell-Hydbom, A., Rylander, L., Giwercman, A., Jonsson, B.A.G, Nilsson-Ehle, P., and Hagmar, L. (2004) Exposure to CB-153 and p,p'-DDE and male reproductive function, *Hum. Reprod.* **19**, 2066-2075.

Rosenberg, M.J., Feldblum, P.J., and Marshall, E.G. (1987) Occupational influences on reproduction: a review of recent literature, *J. Occup. Med.* **29**, 584-591.

Rozati, R., Reddy, P.P., Reddanna, P., and Mujtaba, R. (2002) Role of environmental estrogens in the deterioration of male factor fertility, *Fertil. Steril.* **78**, 1187-1194.

Rudel, R.A., Camann, D.E., Spengler, J.D., Korn, L.R., and Brody, J.G. (2003) Household exposure to phthalates, pesticides, alkylphenols, pbdes, and other endocrine active compounds, *Toxicol. Sci.***72**, 184.

Sanchez-Pena, L.C., Reyes, B.E., Lopez-Carrillo, L., Recio, R., Moran-Martinez, J., Cebrian, M.E., and Quintanilla-Vega, B. (2004) Organophosphorous pesticide exposure alters sperm chromatin structure in Mexican agricultural workers, *Toxicol. Appl. Pharmacol.* **196**, 108-113.

Sheiner, E. K., Sheiner, E., Hammel, R.D., Potashnik, G., and Carel, R. (2003) Effect of occupational exposures on male fertility: literature review, *Ind. Health* **41**, 55-62.

Shih, T.S., Hsieh, A.T., Liao, G.D., Chen, Y.H., and Liou, S.H. (2000) Haematological and spermatotoxic effects of ethylene glycol monomethyl ether in copper clad laminate factories, *Occup. Environ. Med.* **57**, 348-352.

Silva, M.J., Malek, N.A., Hodge, C.C., Reidy, J.A., Kato, K., Barr, D.B., Needham, L.L., and Brock, J.W. (2003) Improved quantitative detection of 11 urinary phthalate metabolites in humans using liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry, *J. Chromatogr. B.* **789**, 393-404.

Silva, M.J., Slakman, A.R., Reidy,, J.A., Preau, J.L. Jr., Herbert, A.R., Samandar, E., Needham, L.L., and Calafat, A.M. (2004) Analysis of human urine for fifteen phthalate metabolites using automated solid-phase extraction, *J. Chromatogr B* **805**, 161-167

Swan, S.H., Elkin, E.P., and Fenster, L. (2000) The question of declining sperm density revisited: An analysis of 101 studies published 1934 – 1996, *Environ. Health Perspect.* **108**, 961-966.

Swan, S.H., Kruse, R.L., Liu, F., Barr, D.B., Drobnis, E.Z., Redmon, J.B., Wang, C., Brazil, C, and Overstreet, J.W. (2003) Semen quality in relation to biomarkers of pesticide exposure, *Environ. Health Perspect.* **111**, 1478-1484.

Takeuchi, Y., Ichihara, G., and Kamijima, M. (1997) A review on toxicity of 2-bromopropane: mainly on its reproductive toxicity, *J. Occup. Health* **39**, 179-191.

Tas, S., Lauwerys, R., and Lison, D. (1996) Occupational hazards for the male reproductive system, *Crit. Rev. Toxicol.* **26**, 261-307.

Tielemans, E., Burdorf, A., te Velde, E.R., Weber, R.F., van Kooij, R.J., Veulemans, H., and Heederik, D.J. (1999) Occupationally related exposures and reduced semen quality: a case-control study, *Fertil. Steril.* **71**, 690-696.

Veulemans, H., Steeno, O., Masschelein, R., and Groeseneken, D. (1993) Exposure to ethylene glycol ethers and spermatogenic disorders in man: a case-control study, *Br. J. Ind. Med.* **50**, 71-78.

Vidaeff, A.C., and Sever, L.E. (2005) In utero exposure to environmental estrogens and male reproductive health: a systematic review of biological and epidemiologic evidence, *Reprod. Toxicol.* **20**, 5-20.

WHO - World Health Organization (1999) *World Health Organization Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, Cambridge University Press, New York.

Whorton, M.D., Milby, T.H., Stubbs, H.A., Avashia, B.H., and Hull, E.Q. (1979) Testicular function among carbaryl-exposed exployees, *J. Toxicol. Environ. Health* **5**, 929-941.

Wyrobek, A.J., Watchmaker, G., Gordon, L., Wong, K., Moore, D., 2nd, and Whorton, D. (1981) Sperm shape abnormalities in carbaryl-exposed employees, *Environ. Health Perspect.* **40**, 255-265.

Xia, Y., Bian, Q., Xu, L., Cheng, S., Song, L., Liu, J., Wu, W., Wang, S., and Wang, X. (2004) Genotoxic effects on human spermatozoa among pesticide factory workers exposed to fenvalerate, *Toxicology* **203**, 49-60.

Xia, Y., Cheng, S., Bian, Q., Xu, L., Collins, M.D., Chang, H.C., Song, L., Liu, J., Wang, S., and Wang, X. (2005) Genotoxic effects on spermatozoa of carbaryl-exposed workers, *Toxicol. Sci.* **85**, 615-623.

Xiao, G., Pan, C., Cai, Y., Lin, H., and Fu, Z. (2001) Effect of benzene, toluene, xylene on the semen quality and the function of accessory gonad of exposed workers, *Ind. Health* **39**, 206-210.

Reproductive Health and the Environment

Edited by

P. Nicolopoulou-Stamati
National and Kapodistrian University of Athens, Medical School, Department of Pathology, Athens, Greece

L. Hens
Vrije Universiteit Brussel, Human Ecology Department, Brussels, Belgium

and

C.V. Howard
Bioimaging Research Group, Centre for Molecular Bioscience, University of Ulster, Coleraine, United Kingdom

Springer

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN-10 1-4020-4828-9 (HB)
ISBN-13 978-1-4020-4828-9 (HB)
ISBN-10 1-4020-4829-7 (e-book)
ISBN-13 978-1-4020-4829-6 (e-book)

Published by Springer,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

www.springer.com

Printed on acid-free paper

Desktop publishing by Dao Kim Nguyen Thuy Binh and Vu Van Hieu.

Editorial Statement

It is the policy of AREHNA, EU–SANCO project, to encourage the full spectrum of opinions to be represented at its meetings. Therefore it should not be assumed that the publication of a paper in this volume implies that the Editorial Board is fully in agreement with the contents, though we ensure that contributions are factually correct. Where, in our opinion, there is scope for ambiguity we have added notes to the text, where appropriate.