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PARENTAL EXPOSURES AND OCCURRENCE OF ADVERSE PREGNANCY OUTCOMES AND CHILDHOOD ATOPIC DISEASES

Linda L Magnusson

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Supervisors:
Dr Helena Wennborg
Karolinska Institutet

Professor Jørn Olsen
University of California Los Angeles
Aarhus University

Professor Bo Lambert
Karolinska lnstitutet

Opponent:
Marja-Liisa Lindbohm
Finnish Institute of Occupational Health

Committée:
Professor Anders Ekbom
Karolinska Institutet

Docent Bodil Persson
Linköping University Hospital

Docent Nils Plato
Karolinska Institutet

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ABSTRACT

The aim of this thesis was to investigate whether parental laboratory work is associated with adverse pregnancy outcomes, and if maternal occupation as well as exposure to organic solvents and smoking during pregnancy are associated with atopic diseases in childhood.

The studies were based on two different study populations. (1) A Swedish source cohort of female and male biomedical laboratory and non-laboratory university employees, 1970-1989. In order to identify pregnancies, outcomes, and additional data, the males were linked to the multi-generation register and their partners, as well as all female employees, to the Swedish Medical Birth Register. Exposure data was based on employee records and questionnaires, sent to research group leaders, concerning the use of agents and techniques. The final study populations consisted of 3003 female pregnancies and 4190 male pregnancies. (2) A Danish cohort encompassing pregnant mothers from Odense and Aalborg 1984-1987. Information on smoking and occupational job titles was collected during pregnancy, and an assessment of organic solvent exposure was performed by occupational specialists. The children were later followed up by questionnaires to parents, at age 14-18, to retrieve information about health parameters such as atopic diseases during childhood. This resulted in a final study population of 7844 children.

Logistic regression analyses did not show any significant associations between female or male laboratory work in general and adverse pregnancy outcomes. However, when analyses were based on specific agents/techniques, maternal organic solvent exposure “periconceptionally” gave an increased odds ratio (OR) of 2.5 and 95% confidence interval (CI) of 1.0-6.0 for major malformations. Maternal work with benzene resulted in increased risk estimates for major malformations, especially neural crest malformations (OR 5.3, CI 1.4-21.1). An increased OR for neural crest malformations was found for paternal work with carcinogens. Paternal work with radioactive isotopes also showed a slightly increased ratio for high birth weight (OR 1.8, CI 1.0-3.2) and altered male/female sex ratio (relative risk 1.2, CI 1.0-1.4). Maternal smoking in pregnancy was associated with wheezing (OR 1.2, CI 1.1-1.5), in a dose response pattern. No association was seen with asthma. Shift work was marginally associated with asthma (OR 1.2, CI 1.0-1.5), and there was a tendency towards an increased risk for asthma and hay fever in association with high solvent exposure, with ORs of 2.0 (CI 0.7-6.1) and 2.6 (CI 1.0-6.9), respectively. Moreover, in an explorative analysis we found elevated risk estimates for a number of occupational groups; e.g. “bakers, pastry cooks, and confectionary makers”, dental assistants, “electrical and electronic assemblers”, “sewers and embroiders”, and “bookbinders and related workers".

In conclusion, the results do not suggest an association between parental biomedical laboratory work in general and pregnancy outcomes. However, specific exposures could be of concern for both female and male employees, most notably maternal organic solvent use. The results on maternal smoking in pregnancy supported an association with wheezing. Certain female occupations in pregnancy might also be associated with childhood atopic diseases, and one potential risk factor could be organic solvent exposure.

LIST OF PAPERS

The thesis is based on the following papers, which are referred to in the text by their roman numerals:

I. Wennborg H, Magnusson LL, Bonde JP, and Olsen J. Congenital malformations related to maternal exposure to specific agents in biomedical research laboratories. *JOEM* 2005; 47: 11-19

II. Magnusson LL, Bonde JP, Olsen J, Möller L, Bingefors K, and Wennborg H. Paternal laboratory work and congenital malformations. *JOEM* 2004; 46: 761-767

III. Magnusson LL, Bodin L, Lambert B, and Wennborg H. Adverse pregnancy outcomes in offspring of fathers working in biomedical research laboratories. Manuscript submitted for publication.

IV. Magnusson LL, Braae Olesen A, Wennborg H, and Olsen J. Wheezing, asthma, hayfever, and atopic eczema in childhood following exposure to tobacco smoke in fetal life. *Clin Exp Allergy* 2005; 35: 1550-1556

V. Magnusson LL, Wennborg H, Bonde JP, and Olsen J. Wheezing, asthma, hay fever, and atopic eczema in relation to maternal occupations in pregnancy. Manuscript submitted for publication.

CONTENTS

LIST OF ABBREVIATIONS

CI	Confidence interval
ED	Endocrine disruptor
ETS	Environmental tobacco smoke
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IgE	Immunoglobulin E
ISAAC	International study of asthma and allergies in childhood
ISCO	International Standard Classification of Occupations
IUGR	Intrauterine growth retardation
LGA	Large for gestational age
MBR	The Swedish Medical Birth Register
NHDR	The Danish National Hospital Discharge Register
NTD	Neural tube defects
OR	Odds ratio
PAH	Polyaromatic hydrocarbons
PCBs	Polychlorinated biphenyls
RR	Relative risk
SGA	Small for gestational age
Th2	T helper 2
TLV	Threshold limit value
VOC	Volatile organic compounds
WHO	World Health Organization

1 INTRODUCTION

Exposures such as nutrition, illnesses, drugs, radiation, and chemical exposure in the workplace or at home may not only constitute a health hazards for parents, but can affect the unborn child. Human reproduction and embryogenesis involves multiple processes such as proliferation, differentiation, migration, and organogenesis, precisely timed events that may be susceptible to environmental insult at different stages. Environmental factors interfering with specific biological processes may consequently induce a range of different developmental effects. These effects may be manifested during pregnancy as pregnancy loss, at the time of birth as adverse pregnancy outcomes, or in postnatal life as functional defects. However, historically, this vulnerable life stage has not been given high priority in human risk assessment (Landrigan *et al.* 2004). Few developmental toxicants have been identified in humans (Schardein *et al.* 1989).

Tobacco smoke exposure is one of the primary environmental health hazards for children during fetal development and childhood (DiFranza *et al.* 2004). Even though the prevalence of smoking among women in reproductive ages has declined in recent decades, smoking remains one of the most important preventable risk factors for pregnancy outcomes (Cnattingius 2004).

The potential for reproductive effects of occupational toxic exposures also represent a significant public health concern (Lawson *et al.* 2003). Exposure to agents such as toxic chemicals and radiation in the workplace is usually higher than in the general environment (Kumar 2004). Occupational exposures are largely preventable, and occupational epidemiological investigations can contribute to the understanding of environmental causes of disease (Blair *et al.* 1996). Still, many environmental and occupational agents are not evaluated for reproductive or developmental toxicity (Lawson *et al.* 2003). Occupational exposure to organic solvents is particularly widespread and represents a suspected health hazard to the offspring since many organic solvents pass the placenta barrier (Kumar 2004). Organic solvents also constitute one of the foremost hazards in laboratory settings, although laboratory work usually involves diverse risk factors of physical, biological and chemical nature.

This thesis focuses on parental exposures such as smoking and occupational exposure, especially to organic solvents and laboratory work, and developmental outcomes such as pregnancy outcomes and atopic diseases.

2 BACKGROUND

2.1 ADVERSE DEVELOPMENTAL OUTCOMES

Developmental toxicity is defined as "the occurrence of adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation." (Selevan *et al.* 2000). Disrupted development may be manifested as death, malformations, growth retardation or functional impairments/disorders (Sadler 2000a). Traditionally, researchers have mainly focused on spontaneous abortions and pregnancy outcomes such as congenital malformations, low birth weight, and preterm birth (Olsen 2000). However, parental exposures may give rise to a range of functional alterations not expressed at the time of birth (long-term health effects) such as asthma, cancer, and neurological or behavioral effects. Along with the increasing industrialization and urbanization, chronic illnesses such as cancer, asthma, learning disabilities, and congenital malformations have become common in western societies (Landrigan *et al.* 1998, Suk *et al.* 2003). Malformations and other defects of development not only cause emotional burden for affected individuals, they also represent a burden for families and society (Kumar 2004). In order to fully identify if an exposure is associated with adverse reproductive effects, several endpoints should be investigated (Savitz *et al.* 1991, Taskinen 1993).

2.1.1 Pregnancy outcomes

2.1.1.1 Congenital malformations

The concept of congenital malformations is not strictly defined, and is often used synonymously with congenital abnormalities, congenital defects, congenital anomalies, and birth defects. These terms usually refer to structural defects that can be observed at birth (congenital) (Kalter 2003). Congenital malformations are commonly classified by severity, for example in major and minor congenital malformations. While major congenital malformations are regarded to be associated with prenatal or perinatal death, surgical or medical care, grave physical handicap, or an extreme cosmetic burden, minor defects are considered to have little or no medical importance (Kalter 2003). Other ways of classifying congenital malformations is by type (usually by ICD - the International Classification of Diseases), etiology (e.g. by single mutant gene, chromosomal abnormalities, discrete environmental, multifactorial, and unknown factors), and pathogenesis (Kalter 2003). A suggested classification based on pathogenesis occasionally used is: *malformation* - a morphological defect of an organ, part of an organ, or a larger region of the body resulting from an intrinsically abnormal development, *disruption* - disturbance of a rudiment which is developing normally, caused by some extrinsic agent, *deformation* - abnormal form, shape or position of a part of the body caused by mechanical forces, *dysplasias* - abnormal organization of cells into tissues and the morphological result. Multiple malformations can further be classified in s*yndromes* -a recognized pattern of malformations with a common cause, and *associations* -a pattern of malformations with unknown cause (Sadler 2000a).

The World Health Organization (WHO) estimates that congenital malformations account for about 3% of child deaths under 5 years of age (Bryce *et al.* 2005). In Sweden, however, malformations are estimated causes of 31-33 % of child deaths before 1 year of age and 12-15% of deaths occurring before 14 years of age (Miljöhälsorapport 2005). Moreover, congenital malformations are a major contributor to health problems and decreased quality of life in childhood (Spencer *et al.* 2003, Wray *et al.* 2005). Around 3-5% live-born infants are born with major structural birth defects (Savitz *et al.* 2002). The majority of defects in Europe are represented by cardiac defects (>25%), followed by limb anomalies (20%), chromosomal anomalies and urinary system anomalies (~15%), central nervous system anomalies including neural tube defects (10%), and oral clefts (7%) (EUROCAT Working Group 2002). The fact that the etiology is largely unknown for malformations such as neural tube defects, cardiovascular system malformations, and oral-facial clefts, which are relatively common and account for a large proportion of morbidity and mortality, is of particular concern (Sever 1994).

2.1.1.2 Birth weight, fetal growth, and gestational age

In 2002, low birth weight and preterm birth occurred with a frequency of about 3.2% and 5.8% in Sweden, respectively (The National Board of Health and Welfare, Statistikdatabaser). Low birth weight, which is usually defined as birth weight <2500g (Kramer 2003), has often been used as marker for perinatal morbidity and mortality. However, the relation between birth weight and these health endpoints is complex (Adams *et al.* 2003). The use of a dichotomous classification of low birth weight is arbitrary and birth weight dependant on when delivery occurs. Preterm birth, is delivery occurring before 37 completed weeks of gestation (WHO 1977, Lemasters 1993). Preterm delivery might be a better health indicator than low birth weight (Adams *et al.* 2003).

Another possibility is to study deviations in terms of mean birth weight. However, paradoxically low birth weight babies show lower mortality in high-risk populations, and according to Wilcox 2001 the residual distribution should instead be estimated (Wilcox 2001). Alternatively, newborns could be classified by their weight for gestational age. A low weight for gestational age (small for gestational age - SGA) is thought to reflect intrauterine growth retardation (IUGR), a reduction in growth before delivery in relation to gestational age. Fetal or intrauterine growth restriction is usually statistically defined when the intrauterine weight is less than 2 standard deviations below the mean for gestational age, while SGA is based on the birth weight below a specific cutoff value, according to standard curves, relative to infants with the same gestational age and gender. Growth deficits may also be classified as symmetric or asymmetric, asymmetric referring to a disproportionate effect on either weight, length or head circumference. Ponderal index is a measure that intends to separate normal from a thin body proportion. Still, IUGR or SGA has been argued not to reflect “truly” growth restricted infants i.e. that did not reach their inherited growth potential, which is considered to serve as an even better indicator of infant health (Bernstein *et al.* 1997). However, no such standard measurement has been developed.

Additionally, high birth weight, large for gestational age (LGA) and postterm birth, may also be associated with a higher mortality, although to a lower extent (Ingemarsson *et al.* 1997, Divon *et al.* 1998, Minakami *et al.* 1999), as well as later health effects (Olesen *et al.* 1997, Ekbom 1998, Hjalgrim *et al.* 2003, McCormack *et al.* 2003, Gunnarsdottir *et al.* 2004, Sin *et al.* 2004, Flaherman *et al.* 2006). The proportion of infants in Sweden with high birth weight (>4500g) and postterm delivery (≥42 weeks) has increased during 1973-2000, and were in 2000 close to 5 % and around 8% (born week 43 or after), respectively (Odlind *et al.* 2003).

2.1.1.3 Other pregnancy outcomes

Spontaneous abortions are pregnancy loss that occurs before the fetus is regarded as an infant. This definition can vary, but is normally in the range of 20-28 weeks (WHO 1977, Lemasters 1993). Later loss will usually be regarded as stillbirths, which can include deaths occurring later than 16-28 weeks of gestation, as well as neonatal deaths within 7 days of births. A change in the male to female sex ratio at birth could also be an indicator of teratogenic or mutagenic events (Källén 1988). Furthermore, the Apgar score is viewed as a predictor of neonatal survival (Casey *et al.* 2001). The Apgar score, assess the condition of the newborn by calculating a score of 0-10 based on five physiological signs of viability: heart rate, respiratory effort, color, muscle tone, and responsiveness to stimuli, where a total score of 7 or higher is used to indicate a good physical condition (Casey *et al.* 2001).

2.1.2 Functional defects

While congenital means present at birth, the terms anomalies/abnormalities/defects and teratology is sometimes also used to incorporate functional impairments (Lau *et al.* 2004). Theoretically, functional defects may involve any biological system including the immunological, hormonal, and neurobehavioral systems. Increasing interest has been directed to the theory of organ or fetal "programming" which suggests that prenatal exposures may have long-term effects on developing tissues and body systems. Programming was defined by Lucas 1991 as "when an early stimulus or insult, operating at a critical or sensitive period, results in a permanent or long-term change in the structure or function of the organism". He speculated that this early event could involve nutritional or other stimulus/insults (Lucas 1991).
The so-called "Barker hypothesis" specifically focuses on the possibility that fetal growth restriction, due to under-nutrition, could cause fetal adaptation and subsequently long-lasting physiological and structural effects (Barker 1998). This hypothesis has stimulated an array of investigations concerning fetal growth/birth weight and potential long-term effects that have suggested a role for programming on a number of adult diseases such as coronary heart disease, hypertension, insulin resistance syndromes and osteoporosis (Nathanielsz *et al.* 2003).
However, evidence is evolving to suggest that many other factors related to intrauterine environment and gene-environment interactions can influence fetal programming and subsequent development of disease (Tantisira *et al.* 2001), including exposure to developmental toxicants (Lau *et al.* 2004). So far, there is a lack of data on environmental influences on children, children's exposure to environmental chemicals, and of low dose exposures on endocrine disruption, asthma and children's health

(Schneider *et al.* 2001). Studies investigating parental exposures in this context are desirable (Kristensen 1999).

2.1.2.1 Atopic diseases

Endocrine and immune regulation plays an important role in normal development and growth (Kristensen 1999). During pregnancy, the maternal and offspring immune system is shifted towards a predominant T helper 2 (Th2) response, probably to ensure a successful pregnancy (Donovan *et al.* 1999, McGeady 2004). Later, this Th2 skewed pregnancy state is normally redirected to a balanced (Th1/Th2) response pattern through immune maturation (McGeady 2004). If this balance is not restored, the child may become predisposed to asthma and atopy, a genetic potential to develop immunoglobulin E (IgE) antibodies in response to antigens (sensitization), which can manifest in different clinical diseases (Maddox *et al.* 2002).

These diseases includes asthma, hay fever/allergic rhinitis, atopic eczema, and gastrointestinal allergies which are characterized by a Th2 phenotype and production of IgE, usually termed atopic allergy or atopic diseases (Terr 2001, McGeady 2004). Asthma is a particularly heterogeneous disease, and the distinction between asthma and its major clinical expression wheezing/wheezy bronchitis can be difficult (Hofhuis *et al.* 2003). This contributes to uncertainty and inconsistency concerning definitions in this area. Many different clinical subtypes have been described in the past. Whether these represent different expressions of a single diseases or different diseases with similar symptoms is not clear (Bel 2004). A recent proposition of different phenotypes is: transient early wheezing, non-atopic wheezing and IgE-mediated wheezing/asthma. Transient early wheezing (wheezing up to 3–5 years of age but not thereafter) seems to be associated with a reduced lung function and subsequent susceptibility to lower respiratory tract illness. Children with non-atopic wheezing, on the other hand, appear to have normal lung function early in life, and develop wheezing in association with viral respiratory agents, especially respiratory syncytial virus (RSV), independently of allergic sensitization, while persistent wheezing, the ‘classic’ asthma, is associated with atopy and atopic markers, early allergic sensitization, significant loss of lung function in the first years of life, and airway hyper reactivity (Stein *et al.* 2004).

Atopic diseases in childhood, is an important public health problem today, with increased prevalence in recent decades in many parts of the world (Taylor *et al.* 1984, Aberg 1989, Hansen *et al.* 2000b, Upton *et al.* 2000, Heinrich *et al.* 2002). Comparatively high prevalence figures are found in western countries (Beasley *et al.* 2000). The apparent epidemic of asthma and allergies during recent decades is most likely attributable to changes in environmental exposures (Johnson *et al.* 2002) and many have tried to link the disease(s) to environmental factors that have increased in the recent decades. Atopic diseases usually have an early onset, which indicate that early environmental factors may influence disease development. Observations of a stronger correlation between infant atopic allergy and maternal allergy further indicate that the intrauterine environment may play a role (Liu *et al.* 2003). The fetus has been observed capable of producing IgE from around 20 weeks of gestation (Jones *et al.* 2000). This suggests that maternal influences, genetic, transplacental or environmental, could affect the development of atopy and asthma (Peden 2000). However, the

possibility of maternal programming of immune response prior to birth, other than by genetic factors, has not been recognized until recently (Warner *et al.* 1998).

2.2 PARENTAL EXPOSURES

It is well known that adverse developmental effects have a multifactorial causality, including both genetic and environmental causes. Nonetheless, for a large proportion of reproductive outcomes, the etiological factors remain obscure (Olshan *et al.* 1993). Many parental exposures could play a role for reproductive health. Examples of confirmed or suspected environmental developmental toxicants include lifestyle factors such as alcohol and tobacco smoke, as well as environmental pollutants such as ionizing radiation, pesticides, organic chemicals/solvents, and biohazards (Figure 1). Environmental pollutants including airborne pollutants such as particles (e.g. diesel exhaust particles), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), volatile organic compounds (VOCs), benzene, formaldehyde, and lead could also be of concern. Many agents can constitute both environmental exposure and occupational exposure, although exposure to factors such as toxic chemicals and radiation are generally higher in the workplace (Kumar 2004).

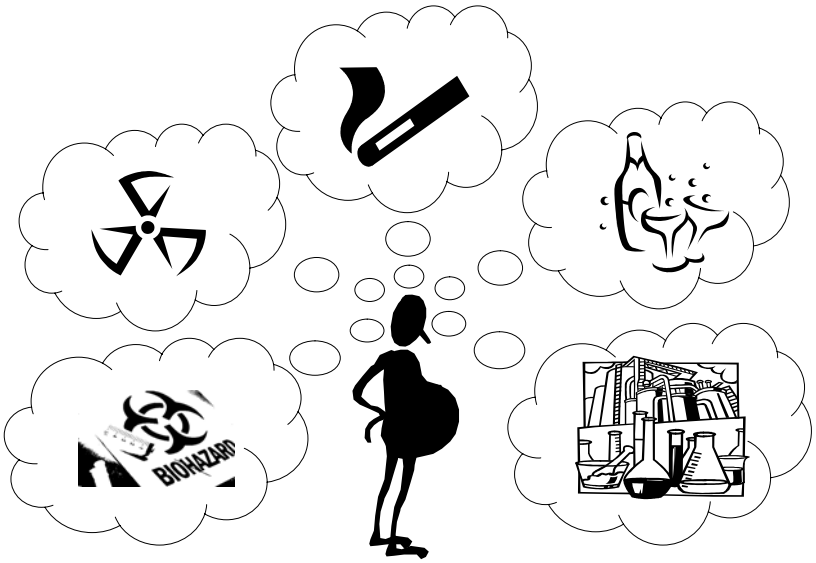

Figure 1. Illustration of some confirmed or suspected developmental toxicants

2.2.1 Tobacco smoke

Mainstream and side stream tobacco smoke contains a broad range of chemicals, over 4000 compounds, although the composition and concentrations in air may differ (Lindbohm *et al.* 2002, Chan-Yeung *et al.* 2003). These chemicals include several agents classified as carcinogens and/or reproductive toxicants. Nicotine, carbon monoxide, polyaromatic hydrocarbons, benzene, toluene, formaldehyde, and nitrosamines are a few examples. A number of these constituents can reach the fetal fluids. Sources of fetal or child exposure to tobacco smoke include maternal smoking, and environmental tobacco smoke from home, work or leisure time activities. Maternal

smoking during pregnancy has decreased during the 1980´s and 1990´s in countries such as the USA, Sweden and Denmark, however, the prevalence remained above 10% in 2000 (Cnattingius 2004).

Smoking has been associated with diminished placental blood flow and subsequent reduced delivery of oxygen (hypoxia) and nutrients to the fetus (Stocks *et al.* 2003). There is convincing evidence that maternal smoking reduces birth weight, with on average 200g (Landau 2001). When data on various reproductive endpoints were summarized, studies also showed consistently increased though modest relative risks for placental abruption, placenta previa, preterm birth, stillbirth, neonatal mortality, oral clefts, and sudden infant death syndrome (Cnattingius 2004). A meta-analysis on passive smoking during pregnancy further suggested that the risk of preterm birth might increase with high exposure (Lindbohm *et al.* 2002). Moreover, fetal exposure to tobacco smoke may be associated with functional disorders such as behavior problems, neurocognitive deficits, and respiratory diseases. However, whether such adverse effects are attributable to prenatal exposure or postnatal exposure to environmental tobacco smoke (ETS) is seldom evident, since women who smoke during pregnancy usually continue to do so after delivery (DiFranza *et al.* 2004).

Whether *in utero* smoke exposure is causally linked to atopic diseases or not is an unsettled matter and published studies have shown conflicting results. Exposure to ETS postnatally has been considered consistently associated with detrimental effects on lung function, respiratory symptoms, and asthma (Health effects of exposure to environmental tobacco smoke. California Environmental Protection Agency 1997, Strachan *et al.* 1998b, Jaakkola *et al.* 2004). In an animal study, ETS exposure influenced the immune response towards a Th2 type, and indicated that smoking may be a Th2 adjuvant (Seymour *et al.* 1997). However, no consistent associations with IgE levels, skin prick positivity, allergic rhinitis, or eczema in children were found in a review by Strachan and Cook 1998 (Strachan *et al.* 1998a).

There are also strong indications of a reduced lung function in children exposed to tobacco in fetal life (Hanrahan *et al.* 1992, Morgan *et al.* 1992, Cunningham *et al.* 1994, Cunningham *et al.* 1995, Stick *et al.* 1996, Dezateux *et al.* 1999), and increased risks for wheezing and asthma in connection with maternal smoking in pregnancy have been suggested (Landau 2001). A study by Schafer et al 1997 reported a significant association between maternal smoking during pregnancy and/or lactation and atopic eczema. Some studies further found *in utero* and/or postnatal smoke exposures to be associated with a higher risk of sensitization (Kulig *et al.* 1998, Kramer *et al.* 2004), while others, did not identify an apparent effect on sensitization (Tariq *et al.* 2000) or atopic eczema (Kerkhof *et al.* 2003). Presence of IgE antibodies in cord blood have also been related to maternal smoking in pregnancy by some authors (Magnusson 1986, Bergmann *et al.* 1995), but not by others (Oryszczyn *et al.* 1991, Ownby *et al.* 1991) or only in infants with a family history of allergy (Atici *et al.* 1995).

The underlying mechanisms for effects of smoke exposure have not been established, however, nicotine has been implicated as a key constituent (Stocks *et al.* 2003). Fetal exposure to nicotine may stimulate nicotine receptors leading to effects on the

dopaminergic system, or airway and alveolar architecture (Sekhon *et al.* 1999, Schuller *et al.* 2000, Sekhon *et al.* 2001).

2.2.2 Occupational exposure

The proportion of women in the workforce in the western world has increased the last 40-50 years, especially in the main reproductive age group (20-35 years) (Chamberlain 1993). In 2003, the female work activity in Scandinavian countries was the highest in the European Union, with an average female activity rate in Sweden of around 75% and nearly 80 % in Denmark (European Commission 2004). Potential occupational hazards to reproduction include work with chemicals, physical and biological agents as well as physical exertion and stress (Kumar 2004). Several suspected reproductive and developmental toxicants, such as heavy metals, organic solvents, pesticides and herbicides, sterilants, and anesthetic gases, are still in commercial and therapeutic use (Lawson *et al.* 2003). Employment sectors with widespread use of such agents are manufacturing settings such as the electronics industry, agriculture, and healthcare (Grajewski *et al.* 2005). Some predominantly female occupations of concern are hairdressers, cleaners, and laboratory technicians (Messing 2004). In Danish industries, high estimated exposure events for carcinogens, reproductive toxicants, allergens as well as neurotoxicants have been found in e.g. manufacture of fabricated metal products, personal services, cleaning, and hair dressing (Brandorff *et al.* 1995).

2.2.2.1 Radiation

Body tissues are easily penetrated by exposure to electromagnetic ionizing radiation, x-rays, and gamma rays, and the exposure delivered to sexual organs and the fetus (Lindbohm *et al.* 2000). Ionizing radiation is highly mutagenic and cytotoxic, and has the capability of inducing DNA damage in germ cells. Epidemiological studies have found maternal exposure to high levels of ionizing radiation in pregnancy to be associated with adverse pregnancy outcomes such as congenital anomalies, growth retardation, and mental retardation, as indicated mainly from studies on survivors of the atomic bombs (De Santis *et al.* 2005). These effects were found to be time and dose dependant. However, no clearly increased genetic risk for different investigated endpoints separately could be detected in the study of atomic bomb survivors (Neel *et al.* 1990).
Findings regarding parental occupational exposure to low doses of ionizing radiation and adverse effects on offspring have been inconsistent. There are few studies on the teratogenic effects of low dose radiation, and no strong evidence for such en effect in women (De Santis *et al.* 2005). Roman *et al* 1996 noted a borderline excess of chromosomal anomalies other than Downs syndrome in children of female radiographers (Roman *et al.* 1996). Conversely, no increased risk was found among children of mothers in the nuclear industry, although there were indications of increased risk for stillbirths and miscarriages (Doyle *et al.* 2000).

Concerning the male reproductive system, adverse effects of ionizing radiation on spermatogenesis have been demonstrated, and animal experiments have added evidence suggesting transmission of paternally-mediated congenital effects (Tas *et al.* 1996, Anderson 2005). One earlier report suggested a genetic effect of low dose preconceptional occupational exposure in fathers (Gardner *et al.* 1990), but subsequent

studies have not demonstrated similar findings (McLaughlin *et al.* 1993, Draper *et al.* 1997, Roman *et al.* 1999). There are also reports of excess of malformations such as neural tube defects and cardiovascular defects (Sever *et al.* 1988, Correa-Villasenor *et al.* 1993, Loffredo *et al.* 2001), and stillbirths (Parker *et al.* 1999), in connection with paternal occupational exposure to ionizing radiation, while other studies have found negative associations (Green *et al.* 1997, Doyle *et al.* 2000). Savitz *et al* 1989 reported a relationship between paternal x-ray exposure and preterm birth (Savitz *et al.* 1989) and Shea and Little 1997 found a weak association with low birth weight (Shea *et al.* 1997b). Paternal occupational exposure to ionizing radiation has also been inconsistently associated with sex ratio alterations (Dickinson *et al.* 1996, Zadeh *et al.* 1997, Hama *et al.* 2001, Maconochie *et al.* 2001).

In view of the contradictory evidence, the role of parental exposures of low doses as those occurring in occupational settings is still controversial (Figa-Talamanca 2000). Moreover, results from studies on maternal exposure to low frequency electric and magnetic fields do not suggest strong adverse effects on development (Lindbohm *et al.* 2000, Shaw 2001, Juutilainen 2003).

2.2.2.2 Physical and psychosocial strain

Physical strain is one of the most common occupational hazards among women (Lindbohm *et al.* 2000). Physically demanding work and prolonged standing have been associated with adverse pregnancy outcomes such as preterm birth and/or SGA (Mozurkewich *et al.* 2000). Shift work can disturb circadian rhythm leading to hormonal disturbances and may be associated with stress (Scott 2000). Exposure to stress activates the hypothalamus–pituitary–adrenal cortex system and the sympathetic nervous system, inducing a release of various hormones (Mulder *et al.* 2002). Published studies have suggested elevated risks for reproductive outcomes such as spontaneous abortions, preterm birth, and low birth weight in association with shift work or night work (Nurminen 1998, Mozurkewich *et al.* 2000). The evidence has, however, been inconclusive and type of shift work as well as mechanisms not clearly elucidated. Exposure to hormones and/or stress during pregnancy has also been related to elevated cord blood IgE (Lin *et al.* 2004) and ability to alter the T helper 1/Th2 balance (Kidd 2003).

2.2.2.3 Biological agents

Among health care and childcare workers, infectious agents can lead to diseases such as toxoplasmosis, listeriosis, German measles (rubella), herpes, chickenpox (varicella), hepatitis B and C, cytomegalovirus infection, parvovirus infection, and HIV infection (Lindbohm *et al.* 2000). Such biological agents could be transmitted to the offspring *in utero* or at delivery. Although the fetus is rarely infected (Ekblad 1995), a serious infection may lead to developmental outcomes such as spontaneous abortions, fetal death, birth defects or preterm delivery (Gilbert 2002).

2.2.2.4 Chemical agents

2.2.2.4.1 Metals

Occupational exposure is the primary source of exposure to lead. Maternal exposure might be associated with an increased risk for spontaneous abortions and reduced fetal growth. High paternal exposure has also been linked to spontaneous abortions, preterm birth, and low birth weight (Bellinger 2005). Moreover, there are indications of an association between parental exposure and malformations, but the evidence is considered to be modest (Bellinger 2005). Additionally, lead has been suggested to cause developmental deficits at low doses (Gardella 2001). Data on other potentially reproductive hazardous metals such as inorganic mercury, cadmium, and nickel is, however, limited (Lindbohm *et al.* 2000).

2.2.2.4.2 Pesticides

The group of pesticides incorporates a variety of chemicals classified into categories such as fungicides, insecticides, nematicides, and rodenticides. Besides the active ingredients, adverse effects of pesticides might also be caused by associated impurities, solvents, carriers, emulsifiers, and other constituents (al-Saleh 1994). Occupational pesticide exposure levels can be higher than environmental exposure. Previous findings give indications of an increased risk for certain reproductive effects such as stillbirths, whereas data on low birth weight have been inconsistent (Hanke *et al.* 2004). Occupational exposure to pesticides could also increase the risk for congenital malformations such as orofacial clefts, limb defects, musculoskeletal and nervous system defects (Garcia 1998, Hanke *et al.* 2004). However, no conclusion have been made due to limitations and controversial observations in the literature (Garcia 1998). Some studies also link paternal exposure to adverse pregnancy outcomes (Savitz *et al.* 1997a, Garcia *et al.* 1998a, Petrelli *et al.* 2000, Ronda *et al.* 2003, Regidor *et al.* 2004), while other studies show a negative association. Certain pesticides are suspected endocrine disrupting chemicals (EDs), which raises concerns about effects of low exposures on cell differentiation, growth, development, metabolism, and reproduction throughout life (Hanke *et al.* 2004).

2.2.2.4.3 Organic solvents

Organic solvents, a diverse group of low molecular weight liquids, are among the most prevalent occupational exposures (McMartin *et al.* 1998, Kumar 2004). They are used extensively in various industries and commercially in degreasers, dry cleaning, paints, fuels, thinners, cleaning agents, glue, and laboratory reagents (McMartin *et al.* 1998, Kumar 2004). Although ubiquitous, more sizable exposure to organic solvents occurs in industrial and laboratory settings (McMartin *et al.* 1998). Organic solvents are readily absorbed through the skin or by inhalation. Many organic solvents also pass the placenta barrier (Kumar 2004). It has been shown that the relative amounts of certain low molecular weight volatile organic constituents in cord blood closely corresponds to quantities present in the maternal blood and that some components are even present in higher concentrations than in the maternal blood (Dowty *et al.* 1976). Some solvents like benzene, toluene, and xylene have also been detected in blood and semen of male workers and an association with lower sperm quality have been indicated (Xiao *et al.*

1999, Xiao *et al.* 2001). Moreover, aromatic hydrocarbons have been observed to increase DNA damage in sperm, and micronuclei in some studies (Migliore *et al.* 2002, De Celis *et al.* 2005). However, the literature concerning organic solvent exposure and adverse pregnancy outcomes has not been entirely consistent and studies have investigated different occupational groups with differences in exposure levels and types of solvents (Taskinen *et al.* 1997).

In a meta-analysis by McMartin *et al* 1998, maternal occupational exposure to organic solvents showed a tendency towards increased risk for spontaneous abortions and a statistically significant association with major malformations (McMartin *et al.* 1998). The meta-analysis was, however, based on only 5 studies, respectively, and the included studies were mainly based on retrospective exposure assessment. The results of a study using biological monitoring supported the hypothesis of a positive association between spontaneous abortion and exposure to organic solvents (Lindbohm *et al.* 1990). Increased risks of spontaneous abortions have also been noted in industrial populations with presumable high exposure (Lindbohm 1995).
Although the link between organic solvents and major malformations was supported by a prospective study (Khattak *et al.* 1999), a clear pattern with respect to type of anomalies observed in different studies have not been apparent. Some specific groups of malformations commonly studied are for example oral clefts, central nervous system defects and cardiac defects including conal or conotruncal malformations (Holmberg *et al.* 1982, McDonald *et al.* 1987, Tikkanen *et al.* 1988, Tikkanen *et al.* 1991b, Tikkanen *et al.* 1991a, Cordier *et al.* 1992, Tikkanen *et al.* 1992, Laumon *et al.* 1996, Bianchi *et al.* 1997, Cordier *et al.* 1997, Garcia *et al.* 1998b, Shaw *et al.* 1999, Brender *et al.* 2002).

Reduced birth weight (Lemasters *et al.* 1989, Ha *et al.* 2002), preterm birth (Wennborg *et al.* 2002), as well as long-term effects such as childhood cancer (Savitz *et al.* 1990a), lower intellectual-, language-, motor-, and neurobehavioral functioning, and vision abnormalities (Till *et al.* 2001a, Till *et al.* 2001b, Laslo-Baker *et al.* 2004, Till *et al.* 2005), have also been suggested. No specific solvent have been observed to be consistently associated with adverse outcome of pregnancy, but particular solvents that have been described as likely to interfere with fertility and pregnancy outcome are tetrachlorethylene, toluene, xylene, benzene, styrene, glycolethers, and other aromatic compounds (Figa-Talamanca 2000). Aliphatic hydrocarbons have also been connected to spontaneous abortions in several studies (Lindbohm *et al.* 1990, Windham *et al.* 1991, Agnesi *et al.* 1997). See also page 13, for specific solvents in laboratory work. However, the results of individual solvents need to be interpreted carefully because of the often complex exposure circumstances.

Paternal occupational exposure to organic solvents and occupations with presumable exposure to organic solvents has been connected to spontaneous abortions (Taskinen *et al.* 1989, Lindbohm *et al.* 1991) and congenital malformations (Olsen 1983, Brender *et al.* 1990, Olshan *et al.* 1991, Correa-Villasenor *et al.* 1993, Schnitzer *et al.* 1995) in some studies, but not in others (Taskinen *et al.* 1989, Kristensen *et al.* 1993, Strucker *et al.* 1994, Blatter *et al.* 1997, Brender *et al.* 2002, Shaw *et al.* 2002). When biological monitoring was conducted there was an increased risk for spontaneous abortions, but not for malformations though the number was small (Taskinen *et al.* 1989). Specific

defects noted include for example neural tube defects, oral clefts, hypospadias, and cardiovascular malformations (Brender *et al.* 1990, Olshan *et al.* 1991, Correa-Villasenor *et al.* 1993, Schnitzer *et al.* 1995). In 2004, Logman *et al* 2004 performed a meta-analysis, which confirmed an association between paternal occupational exposure to organic solvents and central nervous system defects, neural tube defects in particular. The risk of spontaneous abortions was, on the other hand, not significantly increased (Logman *et al.* 2004). Other previous studies have reported alterations in birth weight and/or length in infants of male spray painters or other solvent related occupations (Daniell *et al.* 1988, Hoglund *et al.* 1992), as well as increased risk estimates for small for gestational age (SGA) (Savitz *et al.* 1989, Kristensen *et al.* 1993) and preterm birth (Kristensen *et al.* 1993, Savitz *et al.* 1997b) in relation to paternal solvent exposure. There are, however, studies that did not observe any significant effects of solvent exposure on birth weight or gestational age (Olsen *et al.* 1983, Ha *et al.* 2002). Studies addressing particular solvents or classes of solvents are sparse. Observations in studies on spontaneous abortions have however included exposure to e.g. toluene, miscellaneous organic solvents (including thinners), solvents used in refineries, and in manufacturing of rubber products (Taskinen *et al.* 1989, Lindbohm *et al.* 1991).

The effects of benzene on human reproduction are not clearly elucidated. Benzene is considered a human carcinogen by IARC, and has showed adverse effects such as spontaneous abortions, skeletal abnormalities, and weight retardation in animal models (Ungvary *et al.* 1985, Saillenfait *et al.* 2003). In human studies, maternal exposure to benzene has been linked to spontaneous abortions (Xu *et al.* 1998) as well as shortened gestation, especially in combination with genetic susceptibility (Wang *et al.* 2000), and reduced birth weight particularly in combination with work stress (Chen *et al.* 2000). There are also a few studies addressing paternal benzene exposure, which have found an increased risk for small for gestational age (Savitz *et al.* 1989), but not for spontaneous abortions (Strucker *et al.* 1994), stillbirth, or preterm birth (Savitz *et al.* 1989).

2.2.2.5 Laboratory work

Laboratories constitute a diverse environment where numerous occupational hazards are present (Emery *et al.* 2005). Existing data on chemical exposure in laboratories have indicated that the foremost inhalation hazards are organic solvents such as benzene, toluene, xylene, ethers, dioxane, and carbon disulfide, aldehydes such as formaldehyde and glutaraldehyde, and metals such as mercury (Dement *et al.* 1992). A study by Kauppinen *et al* 2003 concluded that carcinogenic agents such as chromium compounds, carbon tetrachloride, cadmium, and chloroform was also commonly used in different type of laboratories in 1988. Carcinogenic solvents such as carbon tetrachloride, chloroform, and benzene were handled in rather large quantities annually, thus may lead to more substantial exposure than metals (Kauppinen *et al.* 2003). Each laboratory employee was reported as exposed to 2.7 carcinogens on average (Kauppinen *et al.* 2003).

Many laboratory analytical procedures also involve “radio tracing”. Typical amounts of radioactive materials are small. Nonetheless, handling of radioactive materials usually confers a risk for internal organ exposure through inhalation or ingestion (Emery *et al.*

2005). Various chemicals are also used when handling radioactive isotopes. Furthermore, work with biological agents may result in inhalation of biological materials trough dispersion into the aerosol, skin absorption trough contact with contaminated surfaces, or ingestion trough mouth pipetting, food consumption, and animal bites etc. In health care work, rubella, human immunodeficiency virus (HIV), parvovirus B19, cytomegalovirus, varicella, hepatitis B and C are considered of particular concern (Figa-Talamanca 2000), but there are no reliable estimates of the risk of being infected during laboratory activities (Sewell 1995).
Moreover, new substances may constitute novel hazards. Advances in molecular biology have resulted in new techniques involving e.g. genetic modification such as recombinant techniques that may confer hazards for laboratory workers. During genetic modification there may be risks involved when working with the host (virus, bacteria, yeast, fungi, parasite, nematodes, plants, and mammals), the donor organism, or from constituents e.g. retrovirus or bacteria. The exposure levels in laboratories are generally considered to be low (Kauppinen *et al.* 2003, Emery *et al.* 2005). Few concentration measurements are, however, available (Wennborg *et al.* 2001b).

Laboratory employees and their children have been found to have increased levels of sister chromatid exchange in lymphocytes (Funes-Cravioto *et al.* 1977, Lambert *et al.* 1980). A significant increase in genetic damage such as chromatide and chromosome aberrations and micronucleus frequencies have been seen for workers in clinical laboratories exposed to chronic low levels of chemicals (Testa *et al.* 2002), while others have failed to show a significant difference for sister chromatid exchange and micronuclei between laboratory workers and other employees (Narod *et al.* 1988). A recent study did not find a higher frequency of chromosomal aberrations among laboratory personnel in general, except for individuals from a genetic laboratory (Almeida Santos *et al.* 2005).

The evidence on maternal laboratory work suggests an association with adverse pregnancy outcomes, although not conclusively (Dement *et al.* 1992). Maternal laboratory work has been related to spontaneous abortions (Strandberg *et al.* 1978, Kolmodin-Hedman *et al.* 1979, Lindbohm *et al.* 1984, Taskinen *et al.* 1994) in some studies, while negative results have also been reported (Heidam 1984). Furthermore, a slightly increased risk for spontaneous abortions have been found for women who reported to have worked with solvents in laboratories, but the difference was not significant (Axelsson *et al.* 1984). Particular solvents that have been linked to spontaneous abortions among laboratory employees are toluene, xylene, aromatic hydrocarbons (Taskinen *et al.* 1989), and formalin (Taskinen *et al.* 1994). A number of other studies have found increased risk of congenital malformations (Meirik *et al.* 1979, Hansson *et al.* 1980, Ericson *et al.* 1984). Taskinen *et al* 1994 noted alterations in birth weight (Taskinen *et al.* 1994), but did not find an association with congenital malformations; neither did Olsen 1983 and Axelsson *et al* 1984 (Olsen 1983, Axelsson *et al.* 1984). Work with organic solvents has been associated with preterm birth (Wennborg *et al.* 2002). An increased risk for major malformations and preterm birth was also recently reported by Zhu *et al* 2005 for laboratory technicians specifically working with radioimmunoassay or radiolabelling. Work with organic solvents also gave an increased risk for major malformations when an exposure matrix was applied (Zhu *et al.* 2006). Axelsson *et al* 1980 found increased perinatal death rates among

virology personnel (Axelsson *et al.* 1980). Furthermore, indications of increased risks for pre- and postterm births have been seen in connection to work with bacteria (Wennborg *et al.* 2000, Wennborg *et al.* 2002) and an increased risk estimate for LGA among maternal laboratory workers has been observed (Wennborg *et al.* 2000). Paternal employment in "medicine and science" has been associated with decreased birth weight in one study (Savitz *et al.* 1989). In contrast, Shea *et al* 1997 did not find any significant difference in mean birth weight in offspring to this group of employees (Shea *et al.* 1997a).

2.2.3 Methodological issues

Epidemiological studies on reproductive outcomes have often suffered from methodological weaknesses such as exposure and outcome misclassification, small sample size, selection bias, and limited data on potential confounders (Hemminki *et al.* 1995, Lindbohm 1995). The exposure assessment has often been retrospective and self reported which could give rise to recall bias. Underreporting could also be a problem when exposure is self reported (Hemminki *et al.* 1995). Furthermore, adverse outcomes have commonly been related to occupational groups or groups of agents rather than to specific exposures. With respect to paternal occupational exposure, the majority of studies on have only addressed job and industry categories (Tas *et al.* 1996). Thus, many occupational exposure relationships are still controversial and in need of confirmation.

2.3 SUGGESTED PATHWAYS

Possible mechanisms for environmentally induced developmental outcomes can be divided into three main types; genetic, epigenetic and non-genetic mechanisms. Unfortunately, little is known about how these possible mechanisms contribute to different reproductive outcomes in humans (Lindbohm *et al.* 1997). Especially, mechanisms for male-mediated adverse effects on pregnancy are not very well documented (Olshan *et al.* 1993, Tas *et al.* 1996). Conceptually, parental exposure to environmental mutagens can affect germ cells from fetal life until conception of a child. Damage to developing sperm and eggs can occur especially during meiotic division (Lindbohm *et al.* 2000). The genetic mechanism refer to mutations or chromosomal aberrations, whereas the epigenetic mechanism can contribute to a change in the mode of gene expression without changes in DNA sequence, for instance by methylation and parental imprinting (Colie 1993). Non genetic mechanisms can be induced by direct exposure to the fetus trough transfer of exogenous agents cross the placenta or via the amniotic fluid.

Prenatal exposure can induce gene mutations and chromosomal aberrations and has the possibility to interfere with specific biochemical or molecular processes resulting in e.g. cell death, failed cell interactions, reduced biosynthesis or mechanical disruption of tissues (Taskinen 1990). It has also been speculated that there may be a role for male-mediated exposure trough toxic substances in semen (Trasler *et al.* 1999). However, exposure levels of chemicals transferred trough semen probably are considerably lower than the male exposure levels (Klemmt *et al.* 2005). Indirect exposure to the mother and conceptus could also take place if brought home by the father. One specific non genetic mechanism that has received a lot of attention recently is the possibility of

endocrine disruption. Endocrine disruptors are chemicals that can interfere with hormonal signaling systems (Landrigan *et al.* 2003). Agents suspected to be endocrine disruptors include certain pesticides, industrial chemicals and metals. Furthermore, compounds in fuel and some solvents may act as reproductive endocrine disrupters (Reutman *et al.* 2002). A chemical disruption of e.g. the hormonal balance could result in developmental toxicity, neurotoxicity or immunotoxicity (Schneider *et al.* 2001), even at low doses.

2.4 CRITICAL OR SENSITIVE PERIODS

During the last decade there has been an increased awareness of the unique vulnerability to toxic agents that exists in developmental life stages (fetus, young children) (Landrigan *et al.* 2004). Early toxic exposure, including prenatal exposure, appears more likely to result in disease than exposure encountered later (Ekbom *et al.* 1997, Landrigan *et al.* 2002). During development, metabolism and behavior differs, detoxification capacities are immature, and the blood brain barrier is incompletely developed, which can result in higher internal doses (Selevan *et al.* 2000, Landrigan *et al.* 2003). A molecular epidemiological investigation, for example, found higher levels of aromatic and PAH adducts as well as cotinine biomarkers in newborn blood samples, indicative of reduced detoxification capabilities and an increased susceptibility of the fetus to DNA damage (Whyatt *et al.* 2001).

Susceptibility of different developmental processes is, however, dependent upon the timing of exposure. Preconceptionally, the developing gametes may be susceptible to genetic or epigenetic damage (Lemasters *et al.* 2000). For males, the later part of spermatogenesis is probably most sensitive, when DNA is repair deficient (Marchetti *et al.* 2005). Subsequent to implantation, there is a period of differentiation, mobilization, and organization into cells and tissues. This period (organogenesis), which extends from around 3 weeks to 8 weeks of gestation, is the period with greatest susceptibility to malformations (Sadler 2000a). Within this phase the major organs develop. However, each tissue or organ system might have different critical or sensitive periods. One type of stem cells that seem particularly sensitive to environmental insult is the neural crest stem cells (Sadler 2000b). Abnormal migration, proliferation or differentiation of neural crest cells can give rise to neural crest malformations (Sadler 2000a). These include abnormalities in structures such as craniofacial and conotruncal structures (outflow tract and aortic arches), thymus, parathyroid, and thyroid. Sensitivity to neural crest cells is therefore expected during their formation and migration during week 3-4, while general sensitivity to heart development occur during week 3-6 and susceptible periods for the thymus, thyroid and parathyroid glands week 5-8 (Sadler 2000b).

The organogenesis is then followed by a period of growth, histogenesis and maturation with relevance for fetal growth, timing of delivery, and functional capacity of organ systems. The central nervous system with neural development is one potential target for developmental toxicity, for which development start in the embryonic period and extends through adolescence (Rice *et al.* 2000). Furthermore, toxic exposures have a potential to affect lung development, in terms of lung growth and function of the respiratory system, which starts near the end of the first month of gestation (Pinkerton

et al. 2000). Possible targets for toxic insult during gestation include cellular differentiation and branching morphogenesis, although the majority of lung changes continue postnatally (Pinkerton *et al.* 2000). One of the phases that may be especially prone to developmental programming in late gestation or early postnatal life is the alveolar phase (Maritz *et al.* 2005). The definitive gas exchange units, pulmonary alveoli along with the small airways, are formed and mature in this phase (Maritz *et al.* 2005). Moreover, the immune system development occurs largely during prenatal life (Holladay *et al.* 2000). Even though the knowledge about critical windows is incomplete, prenatal windows involving a likely immunotoxic risk include initiation of hematopoesis (week 8 to 10), migration of stem cells and expansion of progenitor cells (week 10-16), and colonization of bone marrow and thymus (week 16 to birth) (Dietert *et al.* 2000). Additionally, maturation and selection of thymic-derived (T) lymphocytes and antibody-producing (B) lymphocytes could be sensitive to toxicant exposure (Dietert *et al.* 2000). Figure 2 presents approximate developmental stages and sensitive/critical windows for certain endpoints.

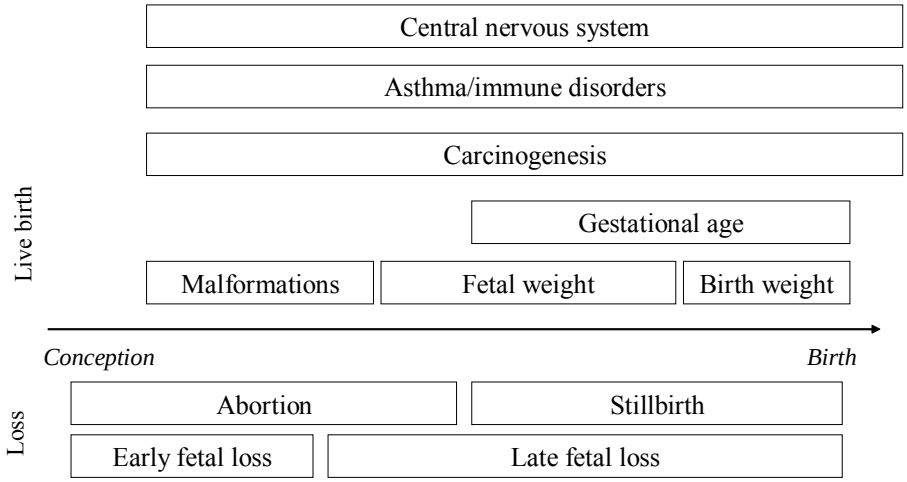

Figure 2. Approximate developmental stages/critical windows for different endpoints. Adopted from Lemasters et al 1993, Pinkerton et al 2000, and Selevan et al 2000

2.5 GENDER CONSIDERATIONS

There are many potential gender differences that can have an impact upon exposure and health effects. Exposure differences can be due to different job tasks, or delivered personal exposure (Messing 2004). Furthermore, there may be gender-specific responses to different toxicants (Lemasters *et al.* 2000). One example is the difference between male and female germ cell maturation. While, female oogenesis starts already during fetal life, spermatogenesis begins in puberty (Sadler 2000a). Oogenesis appear more robust than spermatogenesis to errors and there appear to be sex specific patterns in mutations (Hunt *et al.* 2002). De novo germinal point mutations, structural

rearrangements, and sex chromosome aneuploidies all occur more often during spermatogenesis rather than oogenesis (Chandley 1991, Marchetti *et al.* 2005).

Despite the possibility that paternal factors could contribute to adverse pregnancy outcomes, research has mainly focused on maternal factors. It was first during the 1980's that research investigations generally expanded the focus to male-mediated developmental effects (Lindbohm 1999). Hence, the potential role of paternal exposures has not been extensively investigated and the evidence for male-mediated developmental toxicity still limited (Olshan *et al.* 1993).

Another example of gender differences is the male predominance of asthma and hay fever (Cullinan *et al.* 2003). Boys have been reported to be more susceptible to wheezing/asthma early in life (Anderson *et al.* 1992). This might reflect an increased susceptibility to environmental influences *in utero* or early in life. However, the male predominance could also represent a sex-specific genetic influence by interaction between male sex and parental allergic disease (Melen *et al.* 2004).

3 AIMS

The aim of this thesis was to assess whether there is an association between parental exposures and occurrence of adverse pregnancy outcomes and childhood atopic disease in offspring.

The specific aims were to study the association between:

- Maternal occupational exposure in biomedical laboratories and congenital malformations (Paper I)
- Paternal occupational exposure in biomedical laboratories and congenital malformations (Paper II)
- Paternal occupational exposure in biomedical laboratories and other adverse pregnancy outcomes such as birth weight and gestational age (Paper III)
- Maternal smoking in pregnancy and atopic diseases in childhood (Paper IV)
- Maternal occupations (including shift work) and occupational exposure to organic solvents in pregnancy and atopic diseases in childhood (Paper V)

4 MATERIAL AND METHODS

4.1 STUDY POPULATIONS

The thesis is based on two different study populations:

1. A Swedish cohort of university employees, from laboratory and non laboratory departments 1970-89.
2. A Danish cohort including pregnant women from Odense and Aalborg during 1984-87.

Table 1 gives an overview of the included studies, type of study population used, type of exposure, parent, outcomes, and number of pregnancies considered.

Table 1. Overview of the included studies.

Paper	Study population	Exposed parent	Exposure	Outcomes	N pregnancies/ children*	N pregnancies/ children in analysis†
I	Swedish	Mother	Laboratory work	Major congenital malformations, neural crest malformations	3719	3003
II	Swedish	Father	Laboratory work	Major congenital malformations, neural crest malformations	4760	4170
III	Swedish	Father	Laboratory work	Birth weight (low/high), SGA, LGA, preterm and postterm birth, ponderal index, Apgar score, sex ratio	4760	4190
IV	Danish	Mother	Smoking	Atopic diseases	7844	7811
V	Danish	Mother	Occupation	Atopic diseases	7844	6418

* Total number of pregnancies or children in the study population

† Total number of pregnancies or children considered in the analysis

4.1.1 The Swedish cohort (Paper I-III)

The Swedish source cohort included employees from Karolinska Institutet and the universities of Lund, Linköping, and Gothenburg, Sweden (Wennborg *et al.* 1999). The employees were identified from manual and computerized employee records, with information on age, work tasks, concurrent job titles, calendar time periods, leave of absence for 6 months or longer, change of workplace, and start and end of all employment periods. Eligible subjects were those employed for more than one year and at least 50 % during 1970-1989. In the group of laboratory employees, individuals working in biological or biomedical laboratory departments (N=70) were included, while clinical departments and departments with organic chemistry and physics were excluded. Non-laboratory employees, derived from non laboratory departments such as faculties of Law, Economics, Social Sciences and Science (Mathematics, Statistics etc) (N=34), was used as an internal reference group. Based on job titles and work tasks (job positions), employees were divided into six main employment categories; researchers (including doctoral students), technical personnel, laboratory technicians, animal keepers, maintenance personnel and administrative personnel. In total, the source population consisted of 7958 males and females.

In order to obtain information concerning pregnancies, the source cohort was linked to the Swedish Medical Birth Register (MBR) at the Swedish National Board of Welfare and Health. The linkage to MBR provided information about pregnancies that ended in delivery including stillborns of at least 28 weeks of gestation between 1973 and 2000 (Odlind *et al.* 2003). For the female employees, 3719 births were identified during this period, and 4760 for the male employees. However, to allow linkage to MBR, the male employees (2359 laboratory and 1776 non-laboratory) were first linked to the Multigeneration Register at Statistics Sweden (SCB) to find their female partners and children.

4.1.2 The Danish cohort (Paper IV-V)

The Danish cohort entitled “Healthy habits for two” (Hhft), included all pregnant women in the two Danish cities, Odense and Aalborg during April 1984 to April 1987, who were invited to participate in a health campaign (Olsen *et al.* 1989). At midwife visits, approximately 36th week of gestation, women were given a self-administrated questionnaire about life-style and other social conditions with particular focus on risk behaviors such as smoking, eating, and drinking. About 87% (N=11980) of the pregnant women in the region participated and obstetric information was extracted from medical records. Out of singletons born alive (n=11144), 10636 children and their mothers were still alive, residents in the county, and could be traced by means of the Civil Registration System in 2002. A follow up questionnaire was sent to the parents of these children in order to retrieve information about health parameters such as atopic diseases during the childhood period. The parents, usually the mother, of 74 % of the children responded to the questionnaires. The study population thus consisted of 7844 children of whom 51.6% (n=4045) were boys and 48.4% were girls (n=3798). The cohort and data collection procedure is presented in Figure 1 in Paper IV.

4.2 EXPOSURE

4.2.1 Laboratory work (Paper I-III)

Initially, multiple births, as well as pregnancies that occurred before the start of employment for parents working at laboratory departments, were excluded. Remaining pregnancies of employees from laboratory departments were regarded as exposed to laboratory work in general, after excluding pregnancies of employees working as animal keepers, and maintenance personnel, and of mothers working as technical personnel. Furthermore, offspring of employees working as administrative personnel was considered unexposed. Exposure to specific agents was assessed by means of mailed questionnaires to research group leaders, developed according to recommendations by the International Agency for Research on Cancer (IARC) (Sasco 1992, Wennborg *et al.* 1999, Wennborg *et al.* 2001b). The questionnaire included information about methods and agents, including e.g. 74 different chemicals. The overall response proportion was 66% for all employees. To achieve a homogenous assessment, the research groups were divided into smaller groups if work methods or techniques were considered to differ between participants in a certain group (Wennborg *et al.* 2001b). These methods and agents were classified into the following categories by a group of scientists from different disciplines: organic solvents, carcinogens according to the IARC classification, radioactive isotopes, bacteria, recombinant techniques, as well as work with cell techniques or "animals and cell techniques".

The information about specific exposures was based on 5-year periods; however, as individual changes in work tasks and/or of research groups were recorded, a more detailed exposure assessment could be obtained. To estimate pregnancy specific exposure data, the individual start date for the exposure in question and the timing of pregnancy were used. The exposure periods of interest extended for birth weight and gestational age up to delivery, while for malformations to the end of second trimester of pregnancy as proposed by Shia and Chi 2001 as the end of the acute exposure period of relevance (Shi *et al.* 2001). A long-term model (pre- or periconceptional exposure) considered exposure anytime before that time point, while an acute exposure model (periconceptional exposure) only accounted for exposure subsequent to 1 year before conception (females) or 90 days before conception (males).

4.2.2 Smoking (Paper IV)

Mothers were asked about their smoking habits before being pregnant, in early pregnancy, as well as at the time of filling out the questionnaire, around week 36 of gestation. If the mother reported smoking in early (before week 36), late (week 36) pregnancy or both in early and late gestation, the child was considered exposed to tobacco smoke *in utero*. Mothers who smoked late in pregnancy were further asked about the average number of cigarettes per day and which type and brand of tobacco they smoked. This information was used to classify the nicotine content of the cigarettes based upon reports from the tobacco companies. Smoke exposure late in pregnancy was categorized accordingly in "1-9", "10-19" and "20 or more" cigarettes per day and the level of nicotine as low (<1.5 mg/cigarette), moderate (1.5-1.9 mg/cigarette) or high (≥2.0 mg/cigarette). Moreover, information about parental

smoking anytime after birth was collected in the follow-up questionnaire. If either the mother or father had smoked in this period, postnatal environmental tobacco smoke (ETS) exposure was considered present, provided that the father was recorded as cohabitating with the mother at the time of birth. Based on this information, children were also classified as to whether they had been exposed to smoking in pregnancy only (maternal smoking in late gestation), in the postnatal period only, in both periods or remained unexposed pre- and postnatally.

4.2.3 Occupational exposure (Paper V)

The pregnancy questionnaire provided data on maternal work status during pregnancy. We excluded 1426 children of mothers who reported not to have worked during gestation. Maternal job titles, preconceptional and/or gestational, were recorded on the basis of a Danish occupational classification (Fagkode) (Arbejdsdirektoratet 1979) corresponding to the International Classification of Occupations (ISCO), 1968 version (ISCO) (International Standard Classification of Occupations 1969). Children were classified into "occupational" categories consisting of five digit codes, based on the maternal job titles during pregnancy. If a woman reported a change of job during gestation (n=685), the new job title was used, while the preconceptional job titles were used for women with missing data on this parameter (n=156). The occupational categories were further classified into occupational groups based on the three or four first digits of the codes, and children were classified as exposed to shift work if the mothers recorded that they were shift workers during pregnancy.

Furthermore, three Danish medical specialists in occupational medicine separately performed an assessment of occupational exposure to organic solvents at Danish workplaces in mid 1980'es. The classification of exposure was based upon threshold limit values (TLVs) as they have been found to reflect relative levels of exposure across industries as well as absolute levels (Roach *et al.* 1990). Each occupational category were independently classified as (0) most likely not exposed to organic solvents, (1) most likely exposed at time weighted average levels below the Danish threshold limit value, or (2) most likely exposed at time weighted average levels at or above the Danish TLV, and the final rating was computed as the rounded mean value. Occupations with solvent exposure considered below TLV included occupational groups such as laboratory workers, dental workers, dry cleaners, fitters/solderers, and manufactory workers. Occupations with exposure considered at or above TLV mainly included categories related to painting, printing, furniture manufacturing, and engineering industry.

4.3 OUTCOMES

4.3.1 Congenital malformations (Paper I-II)

The information on congenital malformations was derived from MBR, including all malformations classified according to the International Classification of Diseases (ICD), as well as "significant" and "major" malformations. The category of major malformations was used as the primary outcome of interest. This category excludes certain minor anomalies such as preauricular appendices, undescended testicles,

unstable hip, and nevus (Rylander *et al.* 2002). Craniofacial and conotruncal neural crest malformations such as cleft lip and/or palate, ear effects, thymus aplasia, double-outlet ventricle, Fallot's tetralogy, and ventricular septal defect, were also analyzed as a group, here called neural crest malformations (Hansen *et al.* 2000a). For this purpose ICD-9 or ICD-10 diagnoses were converted to ICD-8 codes. Moreover, neural tube defects (NTD), birth defects occurring in the brain or spinal cord, were investigated in Paper I.

4.3.2 Adverse pregnancy outcomes (Paper III)

Low and high birth weight was defined as a birth weight below 2500g and 4000g or more, respectively. The ponderal index was calculated as the birth weight/ height^3, and low- versus high ponderal index was defined as the lowest and highest 10 % percentiles, respectively. The MBR variable for gestational age is an estimation of gestational age according to a hierarchic method using ultrasound estimation and/or last menstrual period. From this estimation we defined infants born before 259 days (less than 37 weeks) of gestational age as preterm, and those born on day 294 or later (42 weeks or more) as postterm births. SGA and LGA, as defined in the birth register (Marsal *et al.* 1996) and Apgar score (below 7 defined as "low") after 1, 5, and 10 minutes were also considered as well as male to female sex ratio.

4.3.3 Atopic diseases (Paper IV-V)

Responses from the mailed follow up questionnaires were used to categorize children with regard to childhood history of wheezing, asthma, hay fever, and atopic eczema. Children were classified as having asthma, hay fever, and atopic eczema if parents reported that a doctor had diagnosed these diseases at any point in time during childhood. Questions concerning atopic diseases were primarily derived from a Danish questionnaire developed from the UK Party´s questionnaire for atopic dermatitis (Olesen 2001) (Williams 1996). The occurrence of a doctors diagnosis of atopic eczema was also assessed with the question "Have you ever been informed by a doctor that your child has eczema?". Questions on wheezing were also included, which were originally developed for the International study of asthma and allergies (ISAAC) questionnaire (Asher *et al.* 1995). Question number 1 was however used with the modification "Have your child *before 3 years of age* ever had wheezing or whistling in the chest?". Children with an affirmative answer to this question were classified with "wheezing", if they had never been diagnosed with asthma during their childhood. Data on hospitalization in atopic diseases up to 1996 was also retrieved by linkage to the Danish National Hospital Discharge Registry (NHDR) (Andersen *et al.* 1999), which contains information about hospital admissions from 1977 (Yuan *et al.* 2002).

4.4 POTENTIAL CONFOUNDING FACTORS

In Paper I-III, data on potential confounding variables was derived from MBR, including information about mother's age, maternal consecutive pregnancy number, maternal smoking at the first midwife visit, and mother's age. Moreover, maternal chronic diseases as a dichotomous variable were considered, including high blood pressure, epilepsy, diabetes, asthma and lung diseases, kidney disease, as well as systemic lupus erythematosus (SLE). In Paper III, maternal height and gestational

weight gain were also considered. Furthermore, after additional processing, variables such as father's age (by birth year) and maternal occupation were feasible to consider as potential confounders. Information about maternal occupation was derived both from the employee records for female university personnel from the same source cohort as well as from the Medical Birth Register, and was stratified into “biomedical employees”, “health care personnel”, “others” such as other research personnel, students, and employees with potential exposure to chemical, physical and biological agents, “unexposed”, and “unknown” occupation.

As potential confounders in Paper IV-V, we considered a number of potential risk factors or protective factors for atopic diseases that could be imbalanced between groups to be compared. These included variables collected by questionnaires in pregnancy, accompanied with data from medical records, such as maternal education level, maternal age, alcohol consumption, coffee and tea consumption, intake of fish, vitamins/minerals, folic acid, parity, birth weight, gestational age, and gender. Parental socioeconomic conditions were also used and classified in 7 groups based on the parent with the highest rating: group 1 including employees with academic background or with executive jobs, group 2 employees in lower levels of management or employees with an intermediate vocational training like nurses or school teachers, group 3 employees with lower levels of management tasks or workers with a shorter education like policemen or nurse aids, group 4 skilled workers, group 5 unskilled or semiskilled workers, group 6 students, and group 7 others like housewives and unemployed. Moreover, potential confounding factors from the postnatal period that was considered were derived from the follow up questionnaire and included data on breastfeeding, number of children in the household, day care attendance, and animal contact during the first year. In Paper IV, maternal occupation was also taken into account whereas maternal smoking in pregnancy and parental smoking postnatally were considered in Paper V.

4.5 ANALYSES

The primary means of assessing potential exposure-response relationships was by multiple regression analyses. Logistic regression analysis was used to calculate odds ratios (ORs) and 95 % confidence intervals (CIs) for dichotomous outcome parameters such as major malformations, neural crest malformations, low birth weight, high birth weight, SGA, LGA, preterm birth, postterm birth, low Apgar score, wheezing, asthma diagnosis, hay fever diagnosis, and atopic eczema diagnosis. Linear regression models were used to analyze continuous outcome variables (birth weight, and ponderal index), while binary regression was used for analyzing sex ratio. Data analyses were implemented with Stata Statistical Software package 7.0-8.0 (StataCorp. 2003). Certain potential confounders such as mother’s age and previous spontaneous abortions (Paper I), pregnancy order (Paper II) and gender (Paper IV-V) were included in models based on a priori criteria. Other potential confounder covariates were included in the final model by the “backward deletion strategy” (Rothman *et al.* 1998); variables producing a change of the point estimate less than 5-10 % were excluded.

In Paper I-III, analyses was performed both for laboratory work in general and for specific agents, the latter model including all considered agents/techniques. Missing

data concerning smoking habits were grouped as a separate category (no information) in Paper I-II, while in Paper III, missing data with respect to smoking, height, and gestational weight gain were treated by performing imputations. In Paper I-II, adjustment was carried out for consecutive pregnancy number (or pregnancy order), while in Paper III, the strategy for accounting for dependency between pregnancies by the same parent was to adjust confidence intervals for clustering between pregnancies of the same father. The *in utero* smoke exposure (Paper IV) was analyzed both according to number of cigarettes and level of nicotine, adjusted for postnatal ETS exposure (Paper IV). A trend test between number of cigarettes smoked in pregnancy and the endpoints was performed by including smoking as a continuous variable in the logistic regression model. The same procedure was carried out for nicotine level after stratification by number of cigarettes smoked. The results were stratified for birth weight and gestational age, as these covariates are potential intermediate factors (on the causal pathway between smoking and atopic diseases) (Jaakkola *et al.* 2004). Potential interactions between maternal smoking and gender, socio-economic group, and parity were also tested. The analyses on occupational groups were based on the first four digits of the job codes, and included all occupations that had at least 4 cases (Paper V). Clerical workers were used as the reference group. Sensitivity analyses were performed to assess the impact of expert rating differences of organic solvent exposure. For validation, the association between prenatal smoking or occupational exposure and atopic diseases were also estimated based on the data from hospital records.

5 RESULTS

5.1 PAPER I-III

There were no major differences between exposed and unexposed pregnancies (female or male laboratory work in general), except for a slightly higher proportion with mothers who smoked in early pregnancy among non-laboratory pregnancies. Furthermore, the occurrence of previous spontaneous abortions tended to be somewhat higher in exposed pregnancies. The proportion of previous spontaneous abortions was 13.7% in the female exposed pregnancies compared to the 8.5% in unexposed pregnancies. In male exposed pregnancies these proportions were 14.5 versus 11.9, respectively. An assessment of maternal occupation for the male pregnancies also revealed a higher degree of female partners working as biomedical or health care personnel. There was no apparent difference between employees according to response status. Multiple exposures were common both among both females's and male's offspring (see e.g. Table 1 Paper I).

Major malformations were reported with a prevalence of 2.3 % (n=41) in offspring of women with laboratory work before start of the 3rd trimester and 1.9% (n=23; 10 females and 13 males) in the reference group. The corresponding numbers for neural crest malformations were 0.8% (n=14) and 0.7% (n=8), respectively. The most frequent major malformations were congenital heart anomalies and clubfoot. Cleft palate and cleft lip appeared to be more common among exposed pregnancies (3 cases compared to 1), as well as congenital anomalies of the urinary system (6 versus 0 cases), and congenital syndromes affecting multiple organs (4 cases compared to 1 among unexposed).

Among paternal pregnancies, there were 81 major malformations and 21 neural crest malformations altogether. The total proportion between exposed and unexposed did not differ. However, when the malformations were divided by organ system, a slightly higher degree of congenital malformations of ear, face and neck (4 versus 2 cases), urinary system (5 versus 0 cases), clubfoot (12 versus 8 cases), and congenital syndromes affecting multiple systems (3 versus 1 cases) was observed, see Figure 3. The syndromes among exposed included 2 cases of Downs's syndrome and one case of Prader-Willi, while there was 1 case of Downs Syndrome among unexposed.

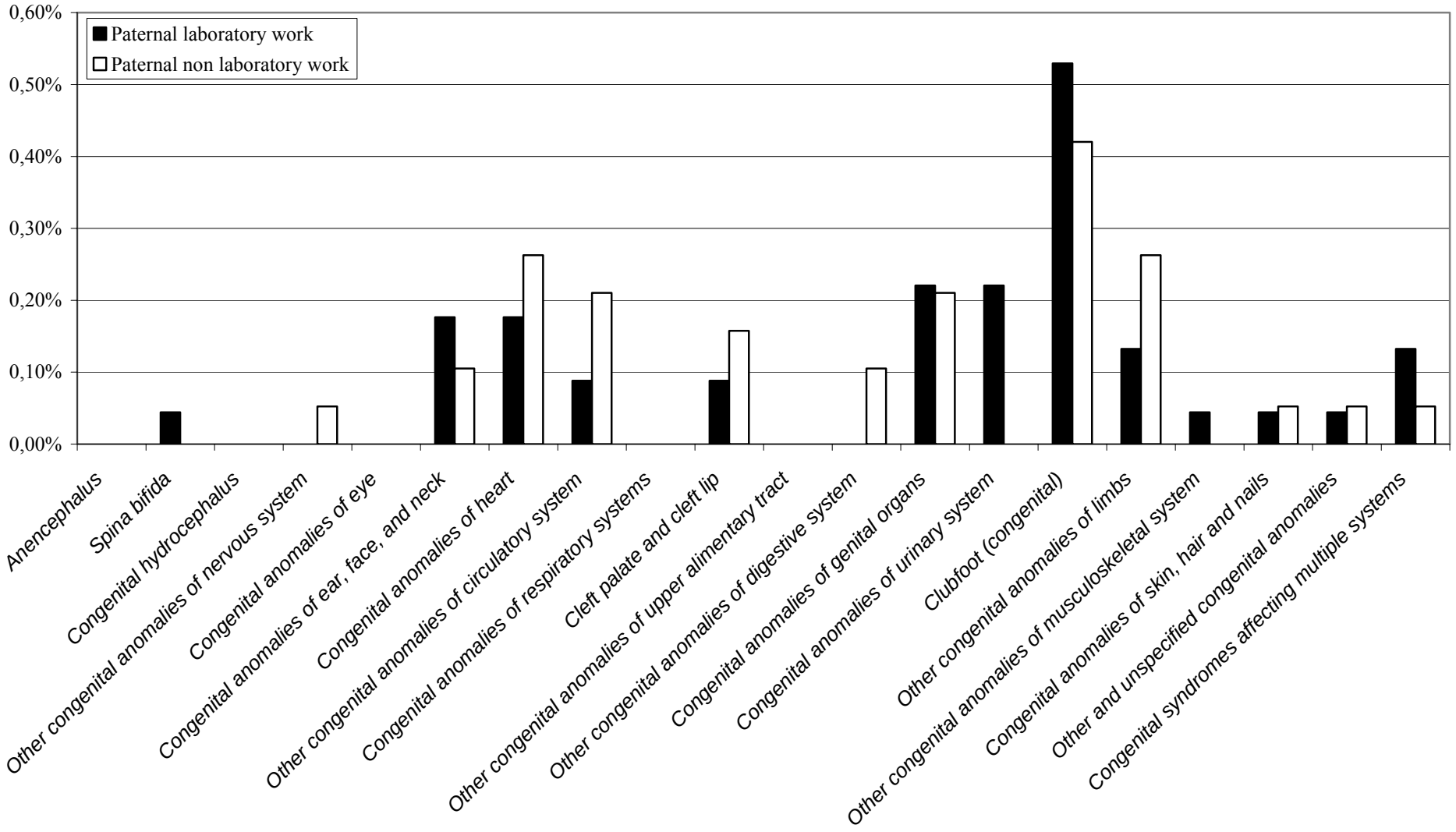

Figure 3. Proportion of pregnancies with major malformations according to organ system and paternal laboratory work

5.1.1 Paper I

The unadjusted odds ratios concerning maternal "laboratory work in general" were for major malformations 1.2 (95% CI 0.7-2.0) with 41 exposed cases and for neural crest malformations 1.2 (CI 0.5-2.9) with 14 cases, respectively. No larger changes were observed after adjustment. The analysis of "current laboratory work" with 23 cases to laboratory employees gave an adjusted OR of 1.5 (CI 0.8-2.8), and for neural crest malformations, 8 cases, an OR of 1.5 (CI 0.6-4.1). Analyses of maternal laboratory work showed slightly increased odds ratios for major malformations, relative to exposure specifically to solvents 2.5 (CI 1.0-6.0), and carcinogens 1.7 (CI 0.8-3.7). Moreover, an OR of 4.5 (CI 1.2-17.5) was noted for neural crest malformations in relation to carcinogen exposure. When specifically investigating benzene, we found a somewhat elevated proportion of major malformations if the mother had worked with benzene any time before the end of the second trimester (3.6%; 10 cases). In pregnancies of women who had worked with other solvents than benzene (n=278/1717), the proportion was 2.5% (17 cases), and among non-exposed pregnancies 1.7% (23 cases), respectively. Long-term exposure for work with benzene gave an OR of 3.5 (CI 1.0-12.0) and 5.3 (1.4-21.1) for current exposure with respect to neural crest malformations. The analyses were adjusted for mother's age at birth, pregnancy number, mother's smoking, and previous spontaneous abortions.

5.1.2 Paper II

Paternal laboratory work in general during 1970-89 was not associated with major malformations in the offspring. The adjusted odds ratios were 1.0 (CI 0.6-1.5) if exposure had occurred anytime before the third trimester and 1.3 (CI 0.8-2.1) if exposure had occurred around conception (periconceptionally). Analyses of paternal exposure to specific agents and techniques showed slightly increased, but not statistically significant, risk estimates for carcinogens, radioactive isotopes, and bacteria, especially when the restricting to periconceptional exposure (Table 3 Paper II). For neural crest malformations, exposure to carcinogens gave an OR of 10.0 (CI 2.1-46.9, 4 cases). An alternative model stratified according to exposure to carcinogenic solvents, other solvents, and no exposure (non laboratory work) periconceptionally indicated increased risk for neural crest malformations for carcinogenic solvents (OR 4.9, CI 1.5-15.8). No clear trend could be observed with respect to multiple exposures (Table 4 Paper II). Adjustments were made for mothers age, smoking, pregnancy order, and previous spontaneous abortions. Moreover, additional adjustment for mother’s occupation and paternal age generated no greater difference in risk estimates.

5.1.3 Paper III

There were no statistically significant odds ratios for pregnancy outcomes concerning paternal laboratory work with pre- or periconceptional exposure. However, we found slightly increased risk estimates for low birth weight (OR 1.2; CI 0.7-1.8), high birth weight (OR 1.1; CI 0.9-1.3), and LGA (OR 1.3; CI 0.8-1.9). When analysis was based on specific exposures, the ratio for high birth weight was somewhat elevated in relation to radioactive isotopes (OR 1.6; CI 1.0-2.4). Only the association between radioactive isotopes and high birth weight was, however, consistently increased with statistical

significance (OR 1.8; CI 1.0-3.2) when restricting the exposure period to periconception. There was no clear trend with increasing number of exposures. Moreover, there was no significant difference in sex ratio, ponderal index, and Apgar score, between exposure groups, except for a slightly higher male/female sex ratio (RR 1.2; CI 1.0-1.4) in relation to radioactive isotope exposure.

5.2 PAPER IV-V

The response proportion was 74%, corresponding to 7844 of the 10 636 children from the present cohort who were possible to trace for follow-up at age 14-18. The number of children for whom asthma had been diagnosed by a physician was 888 (11.4%) according to the parental reports; additionally, 1232 (17.1%) of the children with no asthma diagnosis (n=7205) had wheezing before 3 years of age, and 1083 (13.9%) children had been diagnosed with hay fever. These conditions were more frequent in boys; 12.2%, 17.9%, and 15.3% (vs. 10.6, 16.3, and 12.4% in girls), respectively. The prevalence of atopic eczema, on the other hand, was slightly higher in girls (17.1% compared with 15.1% in boys), with a total proportion of 16.1% (n=1248).

The number of women who reported smoking 'anytime in gestation', according to the questionnaire data from late pregnancy (36th week), was 3252 (41.5%), 417 reported smoking only early in gestation, 12 only late in gestation, and 2813 smoked throughout pregnancy. Smoking in pregnancy was slightly more common among younger mothers as well as in women from lower socio-economic groups.

Of the 6418 children with mothers reporting to have worked during pregnancy, 926 (14%) reported shift work, and 490 (8%) was classified as exposed to organic solvents (470 <TLV and 20 ≥ TLV). Women classified as exposed to solvents more often reported lower educational level, smoking during pregnancy, and generally belonged to lower socioeconomic groups than non-exposed. Women with shift work did not differ considerably from other employees, except for a slightly lower socioeconomic and educational level.

5.2.1 Paper IV

The analysis of prenatal smoke exposure late in pregnancy and wheezing gave an OR of 1.2 (CI 1.1–1.5). In contrast, the risk estimates for hay fever and atopic eczema relative to late *in utero* smoke exposure were 0.8 (CI 0.7–1.0) and 0.8 (CI 0.7–0.9). For wheezing, there was a dose-response pattern in relation to the number of cigarettes the mother smoked per day in late pregnancy (P value <0.01) (Figure 2 Paper IV). The analysis of hay fever and atopic eczema also showed decreased risk estimates in a dose-response pattern with number of cigarettes per day with P values for trend <0.01. There was no association with asthma, and no clear trends for any of the outcome variables were observed according to nicotine exposure. The risk estimates for late gestational smoke exposure were not considerably different when we based our analyses on the hospitalization data only, i.e. the more severe cases. When stratifying for prenatal-, postnatal exposure or both, the results pointed towards a more pronounced association with wheezing for prenatal smoke exposure (OR 1.6, CI 0.7–3.7), compared with parental smoking in the postnatal period only (OR 1.2, CI 1.0–1.4). Adjustments were made for socioeconomic group, maternal occupation, age in pregnancy, coffee

consumption, parity, breastfeeding, gender, and postnatal smoking. Birth weight and/or gestational age did not markedly change the estimates, and did appear to be intermediate factors in this cohort.

5.2.2 Paper V

Shift work was marginally associated with asthma (OR 1.2, CI 1.0-1.5). Furthermore, in the explorative analysis we found elevated risk estimates for a number of occupational groups such as “bakers, pastry cooks, and confectionary makers”, dental assistants, “electrical and electronic assemblers”, “sewers and embroiders”, and “bookbinders and related workers". For these exposures, there was no clear pattern across the different outcome variables. However, there was a tendency towards an increased risk for asthma and hay fever in association with high solvent exposure in pregnancy, with ORs of 2.0 (CI 0.7-6.1) and 2.6 (CI 1.0-6.9), respectively. Analyses were adjusted for socioeconomic group, maternal age, gender, parity, maternal smoking in pregnancy, postnatal smoking by parents, and breastfeeding. The results did generally not differ, when analyses were based on hospitalization data on atopic diseases.

6 DISCUSSION

The present studies did not find any indications of negative effects on pregnancy outcomes from laboratory work in general. However, specific exposures, especially organic solvent use, could be of concern for both female and male pregnancy outcomes. The results on maternal smoking in pregnancy supported an association with wheezing. Certain female occupations in pregnancy might also be associated with childhood atopic diseases, and one potential risk factor could be organic solvent exposure.

6.1 METHODOLOGICAL ASPECTS

6.1.1 Exposure assessment

With exposure information derived from a source cohort of both female and male university employees in the Swedes studies (Paper I-III), we had the opportunity to study both female and male-mediated effects on a number of pregnancy outcomes. Exposure information was primarily collected from employee records, and checked by the group leaders, heads of the department, and in specific cases, also with the individuals themselves. This type of work history information is generally more accurate than union records or interviews and fairly comprehensible (Lemasters *et al.* 1984). However, when analyses were based on all subjects classified exposed to laboratory work in general, some degree of non differential misclassification of exposure can be expected. Non differential misclassification of exposure will generally cause odds ratios to be biased towards the null, which should result in underestimation of the true odds ratio or even in false negative findings.

A strong advantage with the Swedish studies was that we had data on specific agents and methods used by the group members as reported by research group leaders. The exposures were assigned to all members of the groups and in 5-year periods, but in the cases of change of the group or method within this 5-year period, a new exposure information was added for that employee. Misclassification may introduce a problem due to a retrospective exposure assessment, which dated back to 1970. The research group leaders were, however, not aware of the study hypothesis when answering the questionnaire. Thus, recall bias, a differential misclassification of exposure dependant on the outcome, is not likely. We excluded pregnancies to parents who were recorded to be on absence from work periconceptionally, to reduce misclassification due to unexposed periods. Information about the use of protective equipment and industrial hygiene measurements, which can be applied to determine exposure more objectively, was not available. However, even when measurements are available they do not necessarily reflect absorbed dose and account for the dermal exposure route (Lemasters 1993). A great variability in exposures and levels can be expected in research laboratories. For males, the preconceptional stage might be the most critical. Exposures trough semen or “take-home exposure” remain possible, but probably less likely (Selevan *et al.* 1987). Unfortunately, we could not perform comparisons between different exposure windows (spermatogenesis and gestation), which also could have given indications of potential mechanisms involved.

In Paper V, the exposure data was based on the reports of actually held job (job title), as they were stated in the questionnaire by the mothers in the third trimester. Work history information collected by interview has been considered fairly reliable (Baumgarten *et al.* 1983, Stewart *et al.* 1987, Bourbonnais *et al.* 1988). However, when exposures are inferred from job titles, there is an apparent risk for misclassification, since variability of exposure between workers in the groups usually exists. Again, the resulting misclassification is probably non differential and will reduce the possibility of finding exposure-response relations. Nonetheless, this was the best available approach, which has proven useful in the past as an exploratory tool. Furthermore, information on occupation and industry might be the best alternative when dealing with combined and complex exposures (Kogevinas 2003).

Since we had a particular interest in organic solvents, an assessment of organic solvent exposure based on the job titles was also conducted by industrial hygienists. Expert evaluations of job descriptions can in some instances represent the most valid approach (Bouyer *et al.* 1993). The overall agreement between the assessors was fair, but appeared very high between two of the raters. Occasionally, a job exposure matrix can be a useful alternative to expert assessment of exposure in large epidemiological studies. However, job exposure matrices may have low sensitivity and substantial exposure misclassification, especially when used in another country or for purposes other than originally designed for and therefore this was not an alternative in our studies (Roeleveld *et al.* 1993).

In the present study of prenatal smoke exposure (Paper IV), the questionnaire was conducted in a time period when smoking in Denmark was accepted. This probably contributes to a low degree of underreporting. Generally, it is recommended that measurements of cotinine (the metabolite of nicotine) should be made to validate smoke exposure, due to the risk of underreporting of smoking habits among pregnant women. Some investigators, however, argue that even when biomarker measurements are made, they only represent snapshot information and do not take into account changes in smoking habits that can occur during pregnancy (Murray *et al.* 2004). Since, we also have information from two time points in pregnancy we believe that the exposure information might be at least as valid as could have been obtained by commonly used biomarkers. The information was also collected prior to delivery, reducing recall bias. The data on postnatal smoking is, however, rather crude, and the timing of exposure is unknown. Exposure could therefore have occurred after the sensitive periods of lung or immune system development. An advantage with the study was that we also had information about nicotine content of the cigarettes. The nicotine content, however, correlates with other cigarette constituents, contributing to a limited exposure contrast.

6.1.2 Outcome ascertainment

The Swedish Medical Birth Register, used in Paper I-III, is nearly complete; it includes data on 97-99% of all newborn Swedish citizens (Odlind *et al.* 2003). The register is fairly suitable for studies on variables like birth weight, Apgar score etc (Cnattingius *et al.* 1990). Concerning congenital malformations, data is also supplemented from the Registry of Congenital Malformations and a number of secondary registers (Kallen

1987). This system for registration of malformations together identifies around 85-90 % of all malformations observed at birth and neonatally (Ericson *et al.* 1977, Kallen 1987). Major malformations are generally reasonably well ascertained by medical records, except for e.g. congenital heart defects, while minor malformations are less likely to be recorded (Hemminki *et al.* 1995).

Furthermore, MBR does not record information about spontaneous abortions occurring before week 28. A differential survival may lead to a failure to identify certain defects at birth and result in underestimation of true occurrence of those defects. This fact most certainly concern malformations that are fatal such as some forms of aneuploidy or variably fatal such as spina bifida and trisomy 21 (Savitz *et al.* 2002). If the exposure under study is associated with increased risk for spontaneous abortion, an apparent reduced risk for malformations at birth could also be found. Until the second half of 1970´s, screening for prenatal diagnoses was uncommon (Källén 1988), but since screening availabilities have improved, more women also choose elective abortion, which may lead to an under-representation of some malformations. This may concern defects such as neural tube defects, abdominal wall defects, and chromosomal malformations such as Down's syndrome.

In general, the results for malformations were based on few exposed observations. We probably lack power to detect potential risks for uncommon exposures as well as outcomes (specific malformations). Only studying the total malformation rate can fail to reveal an increased rate of specific malformations. However, as in many other cases it was necessary to group the observed malformations. There are many different ways of doing this, but if performed without consideration to heterogeneity in etiology, there will be a loss in statistical power. Conversely, improved statistical power can be achieved if malformations are classified in subgroups that are dysmorphologically more homogenous (Khoury *et al.* 1992). Our approach was to group malformations related to the neural crest. This cell population has been described as vulnerable to environmental compounds (Sadler 2000a).

Since asthma is a heterogeneous and complex disease, no gold standard for defining the condition for epidemiological studies exist (Pekkanen *et al.* 1999). The most generally used definition is parental reports of a history of physician diagnosis (Johnson *et al.* 2002), as used in Paper IV-V. This type of definition is probably one of the most useful (Pekkanen *et al.* 1999), even though it has some limitations. Parental reports of physician diagnoses of asthma has been shown to be accurate (Burr 1992), however, a certain degree of misclassification of outcome data may be expected as parents do not always correctly recall medical diagnoses or if the diagnoses, often given by the family physician were incorrect. Furthermore, the access to medical care may differ leading to potential differential misclassification of medical diagnoses. Misclassification by inaccurate recall of wheezing episodes is also possible. However, in order for this latter misclassification to be differential in Paper V, the mothers should have related the diseases at follow up with their occupation during pregnancy (more than 10 years ago), which is unlikely. Unfortunately, we did not have information about the atopic status, nor if wheezing symptoms were transient or persistent. It is likely, however, that children with wheezing before three years of age, who had not received an asthma diagnosis by age 14-18, mainly represent children with transient symptoms.

6.1.3 Selection bias

The Multigeneration Register is a link between children and biological parents or adoptive parents based on the national registration. The size of paternal discrepancy in the register is not known. When a literature review on paternal discrepancies was performed in 2005, rates varied between 0.8-30%. However, when studies only included populations without already disputed paternity the median discrepancy rate was 3.7% (Bellis *et al.* 2005). Factors such as a lower socioeconomic class (Cerda-Flores *et al.* 1999), younger age and deprivation (trough for example occupational travel) seem to be related to a higher risk of paternal discrepancy (Bellis *et al.* 2005). Considering the relatively high socioeconomic status and scientific knowledge in this laboratory population, we expect the paternal discrepancy in this data to be limited.

It is well known that parental socioeconomic status, educational level etc, can influence participation in interview or questionnaire investigations and health care seeking behavior. Such selection of study subjects may have occurred in the Danish cohort (Paper IV-V). There was a very high participation proportion for the pregnancy questionnaire. However, a selected group of parents might have decided to participate and answer the follow up questionnaire. We could observe that smoking habits differed between mothers in the response and non response group of the follow up questionnaire, however, this difference did not seem to have any greater effect on the effect measures when using the hospitalization data that was available for all.

6.1.4 Potential confounding factors

Pregnancies are not independent events, and adverse pregnancy outcomes are often repeated in successive pregnancies. For this reason, it has been debated whether it is appropriate to adjust for reproductive history. Adjustment would be preferred when attempting to take hereditary factors into account. On the other hand, when the exposure under study could also have been associated with the previous adverse outcome, the exposure-associated risk can be biased by adjusting for this factor (Weinberg 1993). In Paper I-II, when investigating malformations, we chose to include previous spontaneous abortions in the final models, although we did not have actual knowledge of previous exposure. However, the result was not changed when this variable was excluded.

A related aspect is how to treat dependency when more than one pregnancy per parent is included in the study. Pregnancies with the same parents will have a number of background factors in common such as genetic factors and chronic diseases. Different statistical methods have been developed to account for such interpregnancy dependency, and it has been suggested that in some situations the best approach may be to use first pregnancies only (Olsen 1994). In Paper I-II, several pregnancies per parent were used. However, repeating the analyses with only one pregnancy included did not alter the study conclusions. In Paper III, on the other hand, we used “clustering” to take into account the multiple pregnancies included. This did not affect the point estimates but gave slightly wider confidence intervals. Nonetheless, it has been noted that failure to treat each pregnancy independently will only give small statistical errors in most cases (Butler *et al.* 1989).

Other potential confounding factors of importance with respect to pregnancy outcomes are maternal smoking and diseases. These variables were incorporated in MBR from 1983 and onwards. In addition, chronic diseases are probably somewhat underreported in MBR, which could leave residual confounding. However, it seems that the effects of unmeasured confounding from smoking (and other lifestyle factors) will usually be rather weak (Axelson 1989, Kriebel *et al.* 2004) and partial information sufficient for sound conclusions to be made (Axelson 1989). Furthermore, the prevalence of smoking and drinking etc, is likely to be lower among laboratory employees than among other university employees (Wennborg *et al.* 2000, Wennborg *et al.* 2001a), thus confounding causing an overestimation of the exposure association is unlikely. An influence of unmeasured factors can, however, not be excluded.

Moreover, concurrent exposures were common in the laboratories and probably in many other occupations as well. The presence of multiple exposures, underline a need for caution when interpreting the findings concerning specific agents, since the effect measures could be confounded by other agents.

In Paper IV-V, we only have data on two time-points. Consequently our possibilities to adjust for confounding are rather limited. Socioeconomic group is an important factor since it can influence participation in questionnaire investigations and health care seeking behavior. Occupation correlates with sociodemographic, medical and lifestyle factors, and working women have more favorable conditions, for example, are more likely have higher education and income, optimal reproductive age, lower parity, smoke and drink less etc, compared to non-working women ("Healthy worker effect") (Savitz *et al.* 1990b, Moss *et al.* 1993). Unemployed women were therefore excluded from the analyses to reduce differences in sociodemographic and behavioral risk factors. We also adjusted for socioeconomic group, which did not fully explain the results. Nor did the results seem to be explained by factors such as maternal age, life style factors in pregnancy, parity, gender, breastfeeding, daycare attendance, animal contact during first year and postnatal smoking, but there may be other unmeasured and/or unknown factors operating in fetal or postnatal life. Factors such as birth weight and gestational age that are associated with tobacco smoke may lie on the causal pathway. However, this did not seem to be the case in the present data.

Several studies have found estimates for prenatal smoke exposure that differ by family history of atopic/allergic disease (Gilliland *et al.* 2001, Frey *et al.* 2004), although family history had little effect in other studies (Lewis *et al.* 1998, Stein *et al.* 1999). A lower impact of heredity on transient wheezing compared with persistent and late onset wheezing has been observed (Melen *et al.* 2004). Since we lacked data about family history, we cannot exclude potential confounding by that factor, which may be associated with smoking habits. Furthermore, mothers with allergic diseases may avoid certain types of occupations, which could lead to negative confounding by genetic factors, and a tendency to avoid exposures among allergic mothers would probably underestimate any true associations.

Smoking is more common among unskilled manual workers (Lee *et al.* 2004) and shift workers (Knutsson *et al.* 1998). Elevated smoking prevalence's have also been noted in

occupations with exposure to toxic or carcinogenic agents, including metal-exposed and solvent-exposed occupations, as well as among mothers occupationally exposed to second hand smoke (Milham *et al.* 1991). Smoking habits among workers is therefore an important factor to consider in analyses of occupational exposures and reproductive health. We were able to adjust for parental smoking habits, while environmental tobacco smoke from e.g. workplace exposure could not be accounted for.

6.2 FINDINGS

Our findings did not indicate any strong association between laboratory work in general and pregnancy outcomes. However, we did find a few suggestive associations when analyses were based on specific agents and techniques. This supports the view that laboratories should not be considered a single structural unit. Potential reproductive risks are probably confined to specific tasks or departments. Similarly, Zhu *et al* 2006 recently concluded that there was no high risk for reproductive failures in Danish laboratory technicians in general, but they also found an increased risk for specific tasks (Zhu *et al.* 2006).

The finding of an increased risk for major malformations in association with maternal exposure to organic solvents is in agreement with several previous studies (McMartin *et al.* 1998, Zhu *et al.* 2006). The main novel risk indicator in the study of maternal laboratory work and malformations was benzene. Benzene was among the most commonly used carcinogens used during both 1970´s and 1980´s (Wennborg *et al.* 2001b). However, available information on reproductive effects of benzene thus far mainly deals with animal studies.

When studying neural crest malformations as a group, we found an increased occurrence of neural crest malformations in connection with organic solvent exposure. Previous studies have also associated neural crest malformations with environmental factors such as alcohol (Dunty *et al.* 2001, Su *et al.* 2001), which is also an organic solvent. It has been suggested that neural crest malformations may result from damaged neural crest stem cell function or migration. However, a specific chromosome deletion in 22q11 has also been described to cause several syndromes displaying similar patterns of malformations such as DiGeorge and velo-cardio-facial syndromes. A study of chromosome 22q11 microdeletion in conotruncal heart defects found that 93.7% of the deleted chromosomes consisted of de novo mutations, of which two thirds were of maternal origin and one third of paternal origin (Chung *et al.* 2001). It thus seems biologically plausible that both maternal and paternal exposures might contribute to an increased rate of these types of malformations.

Our results suggested an increased risk for neural crest malformations in offspring of fathers working with carcinogens, probably carcinogenic solvents, in laboratories. However, the number of cases in the analysis was low and the confidence intervals wide. Interestingly though, we also noted two cases of Down's Syndrome and one case of Prader-Willi among offspring of fathers working in laboratories. A paternal origin of Down's Syndrome and Prader Willi syndrome trough heritable changes in the genome has been discussed (Colie 1993). Prader Willi syndrome could result from a paternally

inherited microdeletion on chromosome 15q and provides an example of genomic imprinting (Sadler 2000a).

The outcomes of high birth weight, LGA and postterm birth have rarely been investigated. Our observation of an association between paternal exposure to ionizing radiation and high birth weight is in contrast to the weak association with low birth weight found by Shea *et al* 1997 (Shea *et al.* 1997b). On the other hand, similar findings have been reported for female laboratory workers (Wennborg *et al.* 2000). One previous study have also shown a higher proportion of boys in relation to ionizing radiation (Dickinson *et al.* 1996), while others have shown the opposite (Zadeh *et al.* 1997, Hama *et al.* 2001, Maconochie *et al.* 2001). A change in sex ratio could reflect a difference in abortion rates between male and female conceptus or the presence of X-linked recessive lethal genes that selectively kill male embryos (Källén 1988). It has also been proposed that imprinting could play a role in a preferential loss of female embryos (Boklage 2005) and imprinting is implicated in fetal and placental growth. According to a recent study, paternally derived imprinted genes generally appear to promote growth (Isles *et al.* 2005). It has also been suggested that ionizing radiation may induce transgenerational genomic instability mediated trough sperm in an epigenetic manner (Dubrova 2003).

The present study was undertaken under typical conditions in the laboratories 20-30 years ago. An additional cohort study was conducted partly in the same laboratories ten years later, but the results indicated that the agents used were quite similar. The safety management might have been developed since the study period. However, the exposure conditions and agents used, are still relevant in several countries currently undergoing modernization, thus knowledge about the exposures and outcomes studied, can be of significance for risk assessments in other parts of the world.

There is a notion that doses of radiation in the workplace are below those expected to result in adverse developmental effects (Paul 1993). Furthermore, improvements in work place safety have conceivably contributed to a sufficient protection from e.g. radioactive exposure and biological hazards (Figa-Talamanca 2000). The same may be true for organic solvents. However, a recent study on occupational organic solvent exposure suggested that even though protective clothing was used most of the time, it was not enough to protect the developing fetus from neurodevelopmental problems (Laslo-Baker *et al.* 2004).

Despite the fact that workplace exposures most certainly have decreased with increasing use of e.g. safety hoods etc, it is being increasingly recognized that certain exposures could contribute to adverse developmental effects even at low levels. Certain sensitive targets for such effects could involve central nervous system and immune system development. Even subtle effects on functional systems could be sufficient for causing a later onset of disease or may do so in combination with other factors in a causal field (Olsen 2000).

The findings of an increased risk for wheezing, following exposure to tobacco smoke, were consistent with several previous reports (Neuspiel *et al.* 1989, Lewis *et al.* 1995, Strachan *et al.* 1996, Gold *et al.* 1999, Lux *et al.* 2000, Murray *et al.* 2004) and might

be explained by an adverse effect on lung development. Prenatal smoke exposure may affect lung function negatively and give rise to an increased susceptibility towards factors that may provoke wheezing symptoms, such as viral infections or ETS (Martinez *et al.* 1988, Lux *et al.* 2000, Stocks *et al.* 2003). Strachan *et al* 1998 also observed that parental smoking seems to have a stronger effect on non-atopic wheezy bronchitis than allergic asthma (Strachan *et al.* 1998b). Although maternal smoking appears to be associated with transient wheezing, the reduced lung function my last throughout life and increase the risk for chronic lung disease in adulthood (Landau 2001).

Although our possibility to separate the effect of prenatal smoking alone was limited, smoke exposure both *in utero* and postnatally showed a stronger association with wheezing than ETS exposure alone. A negative association, similar to our findings, between maternal smoking in pregnancy and atopy (Soyseth *et al.* 1995, Vonk *et al.* 2004), skin sensitivity (Burr *et al.* 1997), atopic eczema (Soyseth *et al.* 1995, Kuyucu *et al.* 2004), or hay fever in relation to maternal smoking of more than 15 cigarettes/day (Lewis *et al.* 1998), have previously been reported. We also found a more pronounced association if prenatal smoking had occurred in the later part of pregnancy. This is in accordance with the remarks of Landau 2001 that maternal smoking has an effect on all stages of airway development, although the effect upon some of the resulting outcomes appear to be limited is smoking is terminated before the third trimester (Landau 2001). Animal experiments with nicotine exposure also show that adverse effects on the lung development clearly occur during latter stages of lung development (Pierce *et al.* 2002) and have shown interference with the gas exchange regions (Maritz *et al.* 2005). These and other findings have given reason to suspect nicotine as one of the causative agents in tobacco smoke. Animal models have documented many of the same effects on neonates as maternal direct or side stream tobacco smoke exposure by nicotine alone (Pierce *et al.* 2002). Our data did not support a role for nicotine per se, but we had limited power to test the nicotine effect because of small (and perhaps even non-existing) nicotine exposure contrast.

In some cases, the distinction between factors that induce the disease or merely provoke its manifestation is difficult (Cullinan *et al.* 2003). This is the case concerning allergen exposure. While allergen exposure in childhood can increase disease severity, the role of allergen exposure as early as in fetal life is less clear. It is thought that occupational asthma can be used as a model for allergic asthma in the population (Malo *et al.* 2001, Tarlo 2003), since the pathogenesis and pathological alterations are similar. Over 250 work-related agents have been reported to cause occupational asthma and allergy in adults (Chan-Yeung *et al.* 1995). These agents include high molecular weight agents such as flour, laboratory animal proteins, detergent enzymes, that act by inducing IgE mediated responses. However, low molecular weight agents such as diisocyanates, acid anhydrides, aliphatic amines, ethanolamines, heterocyclic and other aromatic amines, metals, aldehydes, reactive dyes are also associated with occupational asthma (Delfino 2002). The mechanism by which these agents induce asthma is less clear. They could act as haptens or by non IgE mediated immunological responses (Tarlo 2003). Recent work related asthma surveillance suggest an additional role for highly reactive low molecular weight chemical sensitizers, mixed contaminant exposures in non industrial settings and respiratory irritants such as cleaning solutions and solvents (Petsonk 2002).

However, few agents associated with occupational asthma or allergy in adults has been linked to respiratory allergic responses in children (Cullinan *et al.* 2003). One example is formaldehyde, which is a volatile organic compound (Delfino 2002). Exposure to VOCs in non industrial settings has been linked to an increased risk of childhood asthma (Rumchev *et al.* 2004). Moreover, maternal exposure to VOC has been associated with an increase in type 2 T cells and a reduction in type 1 T cells in newborns cord blood (Lehmann *et al.* 2002). Animal experiments further suggest that the human immune system is vulnerable to immunotoxicants such as environmental chemicals and ionizing radiation. Adverse effects on immunological or hematopoeitic function have been reported in at least one animal species for benzene, dimethylformamide, lead, PCBs, and pesticides (Schardein *et al.* 1989). Diesel exhaust and tobacco smoke are indicted to be able to shift the immune response towards Th2 response (Peden 2000). Both of these mixtures contain polycyclic aromatic hydrocarbons (PAHs). According to Peden 2000 "It is reasonable to hypothesize that transplacental transfer of Th2 cytokines, transplacental exposure to allergens, or fetal exposure to toxic immunomodulators (such as PAHs) could influence the development of atopy" (Peden 2000).

The analysis of maternal occupations during pregnancy and atopic diseases was explorative and intended to generate hypothesis for future studies. Interestingly, we observed elevated ratios for several occupational groups such as bakers, sewers, and dental assistants that may be exposed to known occupational antigens. However, since multiple comparisons performed, some of the observed associations could have arisen by chance. A putative risk factor for atopic diseases in our study was organic solvents. Agents such as organic solvents and volatile organic compounds might act as immunomodulators. We also noted a marginal association between shift work and asthma, but we could not separate between different types of shift work. Particularly night work has been associated with other reproductive endpoints (Zhu *et al.* 2004a, Zhu *et al.* 2004b).

7 CONCLUSIONS

Taken together, the results do not suggest any association between parental biomedical laboratory work in general and pregnancy outcomes in offspring. However, female laboratory work with organic solvents in research laboratories appears to increase the risk for major malformations. In particular, an association between exposure to benzene and neural crest malformations was indicated. Paternal laboratory work with certain agents such as carcinogens also showed an increased risk estimate for neural crest malformations, and radioactive isotopes for high birth weight as well as altered (male/female) sex ratio.

Concerning long-term consequences of parental exposure in pregnancy, maternal smoking in pregnancy was associated with an excess of wheezing in a dose response manner, which is in agreement with previous studies. There was no association with asthma. Certain female occupations at the time of pregnancy might also be associated with childhood atopic diseases. Exposure to organic solvents tended to be associated with eczema in particular and might be one potential risk factor.

8 FUTURE CONSIDERATIONS

Due to rapid changes in working conditions and exposures, and inconclusive results, continued surveillance of reproductive health of workers handling organic solvents and laboratory workers are warranted. Since the data on benzene is generally limited in humans, our finding of an association between benzene and major malformations need confirmation in future investigations. In order to successfully detect reproductive risks for rare exposures such as specific agents in laboratories and/or outcomes such as specific malformations, large sample sizes will be needed.

Moreover, studies with improved exposure assessment are desirable, focusing on particular exposures as supposed to broad occupational groups ascertained from job titles. Preferably, studies of reproductive outcomes should also increasingly consider the whole reproductive unit including mother, father, and offspring. The evidence for male-mediated developmental toxicity is still limited.

One way of acquiring knowledge of mechanisms of action could involve studies of toxicant effects on gene expression. The epigenetic mechanism may play a role in a variety of processes or toxic responses including fetal growth, placental function, neurodevelopmental processes and immune response.

The effects of nicotine in offspring development need to be clearly elucidated, since information concerning nicotine has implications for safety of smoking cessation. There is also a need for better assessment of the impact of maternal exposure to antigens during pregnancy on later development of allergy in the offspring. If an occupational influence on atopic diseases is confirmed, finding the exposures responsible for these associations as well as potential mechanisms will be future challenges. With particular respect to atopic diseases, studies on the interaction between environmental influences and genetic predisposition could advance our understanding.

Moreover, recent studies have shown inter-generational exposure-disease associations, indicating that our understanding of chronic diseases might benefit from studies with a life course approach (investigating long-term effects of physical and social exposures on chronic disease risk by various factors across an individual's life course but also across generations).

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