The role of medical and illicit drug use in the etiology of birth defects

Epidemiologic studies and methodological considerations

Marleen M.H.J. van Gelder

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Chapter | 1

General introduction

Major birth defects, defined as structural malformations that are of medical, surgical, or cosmetic importance, occur in approximately 2-3% of births.[1] Comparable with cancers, birth defects are a collection of various disorders with each individual defect having its own distribution in the population and its own risk factors.[2] In 2008, 376,000 children younger than 5 years of age died of congenital abnormalities worldwide, which ranks birth defects among the main causes of infant mortality in developed countries.[3] Although a large proportion of major birth defects may be surgically repaired in early life, survivors often have residual disabilities and other long-term morbidities, including an increased risk of cancer.[4] In addition, both male and female individuals with birth defects seem less likely to have children of their own.[5,6] Genetic and non-genetic factors may play a role in the etiology of birth defects, but for the majority of defects the causes are currently unknown.[2,7] Identification of non-genetic, modifiable risk factors for birth defects may facilitate the development of strategies for primary prevention.[8]

In developed countries, 29-99% of pregnant women take at least one prescription medication, depending on the data sources used and the types of drugs included.[9] Although some drugs, such as thalidomide and isotretinoin, are classical examples of human teratogens (traditionally defined as non-genetic risk factors that cause birth defects), the human teratogenic risk is undetermined for over 90% of prescription drug treatments approved for marketing in the United States since 1980.[10] Due to lack of information, pregnant women and their treating physicians are often concerned about the potential risks of the medication for the developing embryo or fetus. As a result, adherence to pharmacological treatment for maternal illnesses, such as hypertension, diabetes, and depression, may be discouraged, which, in turn, may endanger maternal and fetal health. In addition, some women choose to terminate their wanted pregnancies based on fear rather than actual teratogenic effects,[11,12] whereas in other pregnancies fetal development is disturbed by unknown teratogenic exposures that could have been avoided.

Numerous studies have been conducted on the associations between cigarette smoking and alcohol use and the occurrence of birth defects,[13 15] and primary prevention programs often focus on lifestyle modifications to decrease the risks of adverse health outcomes. However, knowledge on the effects of illicit drug use on fetal development is limited, although over 8% of U.S. women report use of one or more illicit drugs during the first trimester of pregnancy.[16] The lack of knowledge may partly be due to the methodological challenges associated with studying illicit drug use during pregnancy. Because of the associated social stigma and false denial of use due to fear of judgment or prosecution,[17] previous epidemiologic studies on the associations between illicit drug use in pregnancy and adverse birth outcomes

most likely suffered from exposure misclassification. In addition, illicit drug use is often accompanied by other potential risk factors, such as poor nutritional status, maternal stress or depression, delayed prenatal care, smoking, and alcohol use,[18] which may have confounded the associations between illicit drug use and birth defects observed.

Objectives and outline of this thesis

Therefore, this thesis aims at obtaining more insight into the teratogenic risks of exposure to medical and illicit drugs during pregnancy. More specifically, the three objectives of this research project were: (1) to assess the influences of medical drug use during pregnancy on the occurrence of major birth defects (Part I), (2) to study associations between prenatal exposure to illicit drugs and major birth defects (Part II), and (3) to evaluate the study methods frequently used in birth defects epidemiology, focusing on the modes of data collection (Part III). Various research methods and several different databases were used to accomplish these objectives.

Part I: Medical drug use

An extensive review of the literature was conducted to provide an overview of the mechanisms through which medical drugs may produce teratogenic effects (Chapter 2). For the medical drugs involved in these teratogenic mechanisms, prescription rates among pregnant Dutch women were estimated using data collected in the IADB.nl database.[19 20] In addition, a systematic review was conducted to give more insight in the current knowledge on the human teratogenic risks of these drugs (Chapter 3). Based on these chapters, two groups of medications were selected to be studied in relation to birth defects in epidemiologic analyses. In Chapter 4, associations between maternal hypertensive disorders and antihypertensive medication during pregnancy and selected birth defects were studied using North American data from the Slone Birth Defects Study.[21] Using data collected in the Norwegian Mother and Child Cohort Study,[22] we assessed the influence of exposure to non-steroidal anti-inflammatory drugs in the first 12 weeks of gestation on the risk of selected birth defects (Chapter 5).

Part II: Illicit drug use

To give more insight in the context in which illicit drugs are being used by men and women of reproductive age, Chapter 6 describes the reproductive health characteristics, risky sexual behaviors, and experiences with sexually transmitted diseases associated with cannabis and cocaine use based on data from the 2002 National Survey of Family Growth in the United States.[23] Following, analyses were performed on data from the National Birth Defects Prevention Study in the United

States[24] to study associations between illicit drug use and adverse pregnancy outcomes In Chapter 7, the characteristics of pregnant illicit drug users are described and the associations between cannabis use and birth weight and gestational age are evaluated Chapter 8 presents the results of a study on associations between illicit drug use and the occurrence of major birth defects

Part III Methodological considerations
Self-reported data are often used in pediatric and perinatal epidemiology, and the need for validation studies is emphasized in Chapter 9 1, in which data from a previous study were reanalyzed In Chapter 9 2, an existing questionnaire on prescription drug use during pregnancy was validated among mothers of infants registered in Eurocat Northern Netherlands [25] Subsequently, the potential influences of exposure misclassification on the effect estimates observed in Chapter 8 for the associations between periconceptional cannabis use and birth defects were quantified in detailed sensitivity analyses (Chapter 10) In a review of the literature, the feasibility of Web-based questionnaires as a new method of data collection in epidemiologic research was evaluated (Chapter 11 1) In addition, reporting practices on the modes of data collection in scientific publications were discussed (Chapter 11 2) To improve on many of the methodological shortcomings of retrospective studies in reproductive epidemiology, Chapter 12 describes the rationale and design of a large prospective birth cohort study, the PRegnancy and Infant DEvelopment (PRIDE) Study

A general discussion of the studies presented in this thesis and future perspectives for research on the associations between medical and illicit drug use during pregnancy and the occurrence of birth defects are included in Chapter 13 1

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Part I

Medical drug use

Chapter 2

Teratogenic mechanisms of medical drugs

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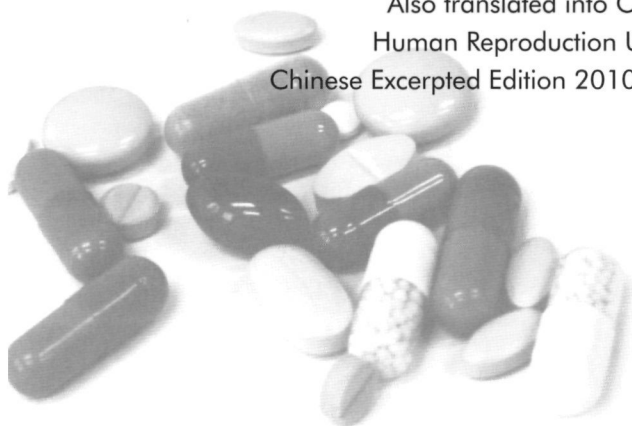
Abstract

Background
Although prescription drug use is common during pregnancy, the human teratogenic risks are undetermined for more than 90% of drug treatments approved in the United States during the past decades. A particular birth defect may have its origins through multiple mechanisms and possible exposures, including medications. A specific pathogenic process may result in different outcomes depending upon factors such as embryonic age at which a drug is administered, duration and dose of exposure, and genetic susceptibility. This review focuses on the teratogenic mechanisms associated with a number of medications.

Methods
We used three methods to identify the teratogenic mechanisms of medications: the MEDLINE and EMBASE databases, two recent books on teratogenic agents, and a list of drugs classified as U.S. Food and Drug Administration class D or X. Mechanisms were included only if they are associated with major structural birth defects and medications that are used relatively frequently by women of reproductive age.

Results
We identified six teratogenic mechanisms associated with medication use: folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption, and specific receptor- or enzyme-mediated teratogenesis. Many medications classified as class X are associated with at least one of these mechanisms.

Conclusions
Identifying teratogenic mechanisms may not only be relevant for etiologic and post-marketing research, but may also have implications for drug development and prescribing behavior for women of reproductive age, especially since combinations of seemingly unrelated prescription and over the counter medications may utilize similar teratogenic mechanisms with a resultant increased risk of birth defects.

Background

Since approximately half of the pregnancies in the USA are unintended,[1] many women expose their unborn children to drugs before they know they are pregnant Furthermore, prescription drug use is common during pregnancy in many other countries as well, with prevalence estimates ranging from 44% to 79% in several European countries [2 4] Because pregnant women were often excluded from clinical trials and data from animal studies are not always predictive for a teratogenic effect in humans, drug use by pregnant women can be considered experimental in most instances Nevertheless, the use of medication is sometimes inevitable in the treatment of women of reproductive age and during pregnancy Although it has clearly been shown that some drugs, e g , thalidomide and isotretinoin, can produce birth defects, the teratogenic risks in human pregnancy are undetermined for more than 90% of drug treatments approved in the USA in the last decades [5 7] Birth defects are the leading cause of infant mortality and the etiologic pathways are largely unknown for many defects A particular birth defect may be caused by many different factors (e g , genetics, environmental agents, medications, physical conditions) as well as by different mechanisms, whereas a specific pathogenic process may result in different outcomes for chemical or drug exposures depending upon such factors as embryonic age, duration and dose of exposure, and genetic susceptibility [8 9] In addition, maternal determinants, including drug administration, distribution, metabolism, and excretion, may also play an important role Although the mechanisms by which drugs may cause birth defects are still not completely understood, we will present an overview of the most important teratogenic mechanisms known today Identifying these mechanisms may be relevant for drug development, (post-marketing) research, and prescribing of medications to women in their reproductive years

Methods

We used three methods to identify the most important teratogenic mechanisms associated with medical drug use First, in January 2009, the MEDLINE and EMBASE bibliographic databases were used as search engines employing a combination of keywords, including 'birth defects', 'congenital abnormalities', 'mechanism', 'teratogenesis', 'abnormalities, drug-induced', 'pregnancy', and 'pharmaceutical preparation' Only articles that were published in the English language were included Secondly, two recent books on teratogenic agents by Shepard and Lemire[10] and Schaefer et al [9] were hand-searched for additional mechanisms Finally, all medications classified by the U S Food and Drug Administration (FDA) as class D ("the potential benefits from the use of the drug in pregnant women may be

acceptable despite its potential risks") or class X ("contraindicated in women who are or may become pregnant")[11,12] were screened. Only mechanisms producing major structural birth defects associated with medications that are relatively frequently used by women of reproductive age (defined as an annual prescription rate of >0.5%, if known) were included in this review. These mechanisms are folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption, and specific receptor- or enzyme-mediated teratogenesis. It should be noted that, so far, some of these mechanisms are principally understood from animal models; however, these mechanisms may produce birth defects in humans as well. In addition, some drugs may be involved in multiple mechanisms for producing birth defects.

Folate antagonism

Folate, the generic term for a water-soluble B vitamin, occurs in high concentrations in certain natural foods (fruits, leafy green vegetables, beans and liver) as polyglutamate. The synthetic form, folic acid (a monoglutamic acid), is used in food fortification and vitamin preparations. Folic acid has a higher bioavailability than food folate.[13] Folate is converted through two reduction reactions by dihydrofolate reductase (DHFR) to the naturally bioactive form tetrahydrofolate (THF), which is converted into 5-methyltetrahydrofolate (5-MTHF) monoglutamate. 5-MTHF is the main form of folate in the blood circulation and is transported into cells by three routes: by membrane-associated receptors, by a carrier-mediated system, the reduced folate carrier, and by passive diffusion.[14,15] Inside the cell, it acts as an essential co-enzyme in many biochemical reactions by being an acceptor or donor of one-carbon units in, for example, purine and pyrimidine synthesis and DNA methylation reactions (Figure 2.1). Since rapidly proliferating tissues require DNA synthesis the most, it is obvious that folate-dependent reactions are essential for fetal growth and development and that folate requirements increase during pregnancy. In addition, DNA methylation is known to be involved in the epigenetic control of gene expression during development.

Several drugs disturb the folate metabolism and may have a teratogenic effect through inhibition of the folate methylation cycle (Table 2.1). Two general groups of drugs act as folate antagonists. The first group consists of competitive inhibitors of DHFR and includes methotrexate, sulfasalazine, triamterene, and trimethoprim, which block the conversion of folate to THF by binding irreversibly to the enzyme.[16] They are used in the treatment of a variety of diseases, such as inflammatory bowel disease, rheumatoid arthritis, hypertension, and urinary tract infections. The second group of drugs may antagonize other enzymes in the folate metabolism, impair
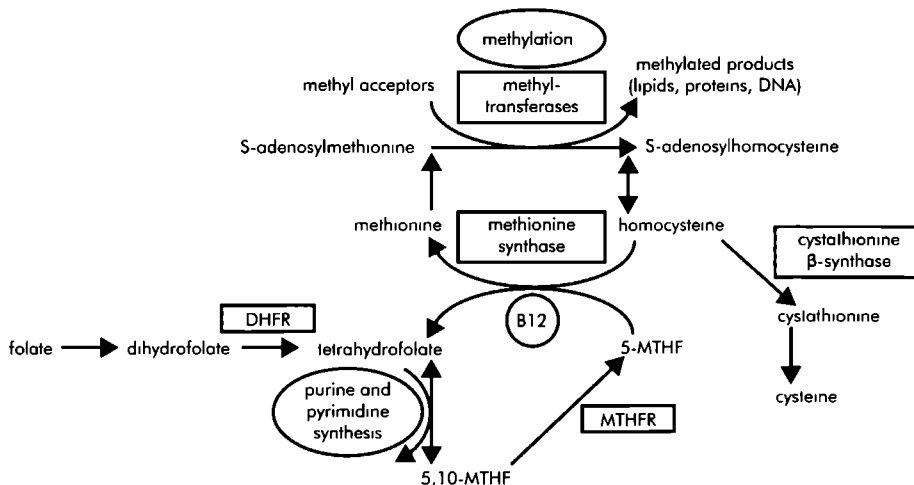
Figure 2.1 Folate-homocysteine-methionine metabolism B12, vitamin B_{12}; DHFR, dihydrofolate reductase; MTHF, methyltetrahydrofolate; MTHFR, methyltetrahydrofolate reductase

Table 2.1 Medical drugs associated with folate antagonism.

Medication	Main indication	Interference with folate metabolism
Carbamazepine	Epilepsy, bipolar disorder	Impairment folate absorption
Cholestyramine	Hypercholesterolemia	Impairment folate and vitamin B_{12} absorption
Cyclosporine	Transplants, psoriasis, atopic dermatitis	Possible interference folate dependent remethylation
Lamotrigine	Epilepsy, bipolar disorder	Inhibition DHFR
Metformin	Diabetes	Interference vitamin B_{12}
Methotrexate	Cancer, some auto-immune diseases (rheumatoid arthritis, psoriasis)	Inhibition DHFR
Nicotinic acid	Hypercholesterolemia	Decrease activity CBS
Phenobarbital	Epilepsy	Impairment folate absorption
Phenytoin	Epilepsy	Impairment folate absorption, decrease activity methionine synthase, possible decrease activity MTHFR
Primidone	Epilepsy	Impairment folate absorption
Pyrimethamine	Malaria	Inhibition DHFR
Sulfasalazine	Inflammatory bowel disease, rheumatoid arthritis	Inhibition DHFR
Triamterene	Hypertension, edema	Inhibition DHFR
Trimethoprim	Urinary tract infection	Inhibition DHFR
Valproic acid	Epilepsy, migraine headache	Antimetabolite of folate

CBS, cystathione β-synthase, DHFR, dihydrofolate reductase; MTHFR, methyltetrahydrofolate reductase

folate absorption, or increase folate degradation. This group primarily consists of anti-epileptic drugs, including valproic acid, carbamazepine, and phenytoin. The teratogenicity of folate antagonists in humans was first suggested by reports of women who were given aminopterin in the first trimester of pregnancy to induce abortion.[17] Some anti-epileptic drugs, e.g., carbamazepine and valproic acid, are generally known to increase the risk of folate-sensitive birth defects, such as neural tube defects, orofacial clefts, and limb defects. So far, only three studies have been conducted to determine the effect of folate antagonists as a group on the occurrence of birth defects in humans, but the results are inconsistent, particularly for DHFR inhibitors.[18 20] In addition, polymorphisms in genes associated with the folate metabolism, including methylenetetrahydrofolate reductase (MTHFR),[21 22] methionine synthase reductase (MTRR),[23] and methylenetetrahydrofolate dehydrogenase (MTHDF1),[24] may lead to differences in the susceptibility of individuals to folate antagonists.

Experimental studies in a number of animal species demonstrated that folate deficiency causes intrauterine death, growth retardation, and various congenital malformations.[25 26] The fact that folic acid supplementation in the periconceptional period decreases the risk of neural tube defects in humans[27] strongly suggests a causative role of folate deficiency in the etiology of these defects. Recently, low blood folate status has been associated with an increased risk of neural tube defects.[28 29] Besides folate deficiency, a low maternal vitamin B_{12} (cyanocobalamin) status has also been shown to be an independent risk factor for neural tube defects.[30 31] Vitamin B_{12} is cofactor to methionine synthase, which converts homocysteine into methionine. Therefore, a shortage of vitamin B_{12} also leads to a distorted folate metabolism.

The exact mechanism by which disturbances of the folate metabolism increase the risk of neural tube defects is unclear. Women who carry a fetus with a neural tube defect have significantly higher levels of homocysteine in plasma and amniotic fluid than control subjects,[32 33] which may be caused by folate deficiency. Several hypotheses have been proposed to explain how increased levels of homocysteine, or the accompanying decreased methionine levels, could cause neural tube defects. First, homocysteine itself may be teratogenic during the neurulation process, causing dysmorphogenesis of the neural tube, heart, and ventral wall in chick embryos.[34] In rat and mouse embryos, however, increased homocysteine levels did not cause neural tube defects.[35 36] Therefore, it seems that elevated plasma homocysteine levels itself may not cause neural tube defects, but are a biomarker of disturbances in the methylation cycle which may result in neural tube defects. More likely, intracellular accumulation of homocysteine leads to increased levels of S-adenosylhomocysteine,

which is a competitive inhibitor of many methyltransferases, through which gene expression, protein function, and the lipid and neurotransmitter metabolisms might be dysregulated.[15 37] Furthermore, the decreased remethylation of homocysteine to methionine leads to decreased levels of S-adenosylmethionine, which is the most important methyl-group donor in the methylation cycle. As a result, neurulation could be disturbed by inadequate gene and amino acid methylation.[15] Methylation steps also play an important role in the metabolism of lipids and neurotransmitters and in detoxification of exogenous substances. This stresses the crucial role of the folate metabolism for normal cellular function, especially during cell division and differentiation. This hypothesis is supported by previous studies showing that methionine is required for normal neural tube closure in rat embryos.[38 39] Disturbances in folate metabolism are also thought to play a role in the etiology of orofacial clefts,[40 42] heart anomalies,[43 44] limb reduction defects,[40 43 44] anal atresia,[45] and urinary tract anomalies[40 43] since folic acid supplementation, alone or in multivitamins, seems to have a protective effect on the occurrence of these birth defects, although the evidence is not as strong and consistent as for neural tube defects. Therefore, it seems likely that medications that act as folate antagonists may cause various birth defects through similar mechanisms.

Neural crest cell disruption

The neural crest is an important, pluripotent cell population that originates in the neural folds. The neural crest cells can be divided into two major populations: the cranial and truncal neural crest. During neurulation, the neural crest cells detach from the neural folds and migrate into the embryo to give rise to numerous structures. In the craniofacial region, various cell types and structures, including intramembranous bone, cartilage, nerves, and muscles, are derived from the cranial neural crest. The truncal neural crest produces important components of the peripheral nervous system.[46] The cardiac neural crest is a subpopulation of the cranial neural crest, which migrate into the cardiac outflow tract to mediate septation and into other derivatives of the pharyngeal arches, such as the thymus and the thyroid and parathyroid glands.[47] Therefore, neural crest-related cardiovascular malformations include aortic arch anomalies and conotruncal defects.[48] Membranous ventricular septal defects are also neural crest-related, since the membranous part of the interventricular septum originates from the cardiac neural crest, whereas the muscular part originates from the mesenchyme.[49] Non-cardio-vascular defects that have been proposed to be neural-crest related are craniofacial malformations,[50] esophageal atresia,[51 52] and abnormalities of the pharyngeal glands.[53]

Proper induction, migration, proliferation, and differentiation of neural crest cells are tightly regulated A variety of molecular signals and receptors are implicated in neural crest cell development Fibroblast growth factors may be involved in the induction of neural crest cells [54] Integrins, a family of cell surface receptors, play a role in the interaction of neural crest cells with the extracellular matrix,[55] whereas interactions between neural crest cells are mediated by cadherins [56] It has been suggested that Pax3 is necessary for the fine tuning of the migration process of cardiac neural crest cells [57] Endothelins and their receptors may be required for the migration, differentiation, and proliferation of neural crest cells [58 59] Therefore, drugs that interfere with these molecular pathways, such as bosentan,[60] which is indicated for the treatment of pulmonary hypertension and to reduce new digital ulcers associated with systemic sclerosis, may induce neural crest-related malformations In addition, *in vivo* and *in vitro* experiments suggested that altering levels of folate and/or homocysteine cause abnormalities of cardiac neural crest cell migration, differentiation, and cell cycle progression,[61] thereby connecting this teratogenic mechanism with folate antagonism However, one of the most important signaling molecules in neural crest cell development is retinoic acid, the biologically active form of vitamin A Excesses[62] as well as shortages[63] of retinoic acid seem to cause neural crest-related malformations, indicating that proper retinoid homeostasis is necessary for normal development Embryonic retinoic acid synthesis and degradation are performed by retinal dehydrogenases and CYP26, respectively [64 65] In addition to retinoids used in the treatment of dermatologic conditions, such as tretinoin, isotretinoin, and etretinate, other drugs that inhibit these enzymes may also be involved in disturbances of retinoid homeostasis It has been suggested that retinoid teratogenicity is mediated by the retinoic acid receptors (RARs) and retinoid X receptors (RXRs) [66] These nuclear ligand-inducible receptors are transcription factors themselves and affect other downstream genes that are important in development [67] This hypothesis is strengthened by the fact that mice lacking RARs and RXRs show developmental defects similar to those caused by vitamin A deficiency, including neural crest-related malformations [68 69] Alternatively, increased *Hox* gene expression may underlie the detrimental effects of excess retinoic acid on the development of structures derived from the neural crest [70 71]

Endocrine disruption: sex hormones

Since the 1940s, a number of drugs have been developed to mimic or inhibit the actions of hormones, including diethylstilbestrol (DES), oral contraceptives, and hormones used in fertility treatment These medications and other endocrine disrupting chemicals (EDCs), such as bisphenol A and phthalates, may interfere with the physiologic functions of endogenous hormones by affecting their release,

binding, or metabolism Their actions may not only depend upon their affinity or specificity for the estrogen and/or androgen receptors, but also upon their ability to activate or inhibit receptor-mediated actions, which are dependent upon the absorption, distribution, metabolism, and excretion (ADME) of these molecules as well The actions of EDCs *in utero* have been of concern because of their possible impact on the developing reproductive systems, especially since treatment of pregnant women with the synthetic estrogen DES led to an increased risk of vaginal adenocarcinoma in their daughters [72] Since human effects were identified first, animal studies have been conducted to confirm these clinical observations and to investigate the differences between synthetic and natural estrogen actions on the embryo or fetus [73 74] It is well known that human sex hormone-binding globulin has a substantially higher affinity for estradiol than for DES or other synthetic hormones,[75] which suggests that DES may be more readily available to cross the placenta DES is also metabolized to reactive intermediates which covalently bind,[76 77] whereas estradiol is not metabolized to similar reactive intermediates [78 79] In addition, α-fetoprotein binds estradiol but not DES [80] So besides the capability of the placenta to reduce the transfer of estradiol, plasma binding and metabolism of this endogenous hormone to less active estrogens may be important defense mechanisms for the fetus to reduce the actions of estradiol, which are apparently not available for the synthetic estrogen DES

Besides an increase in the risk of vaginal adenocarcinoma in daughters, prenatal exposure to DES has also been associated with an increase in reproductive disorders in sons[81] and grandsons [82 83] In male animals, prenatal exposure to EDCs with estrogenic or anti-androgenic properties have been shown to cause hypospadias and cryptorchidism [84 86] In addition to drugs that influence endocrine homeostasis as their primary mechanism of action, coatings for oral medications, such as mesalamine and omeprazole, may be a source of EDC exposure [87] These enteric coatings contain phthalates, which may affect human male reproductive development due to their anti-androgenic properties [88] Additionally, other preparations may contain phthalates as plasticizers,[89] but it should be noted that phthalates do not bio-accumulate and are excreted rapidly in contrast to some other EDCs The susceptibility to EDCs may also vary greatly between individuals due to genetic factors [90] Therefore, it is questionable whether the levels of phthalates in medications in particular are high enough to produce male reproductive tract anomalies in humans In epidemiologic studies, omeprazole and mesalamine have not been associated with an increased risk of major birth defects [91 92]

Male development is more susceptible to endocrine disruption than female development because of its hormone dependence [93] However, since synthetic

hormones and EDCs may affect endocrine homeostasis in multiple ways, the underlying teratogenic mechanisms are often difficult to unravel. Because of considerable species differences and markedly different estrogen levels in normal human pregnancy compared with normal rodent pregnancy, it is debatable whether certain mechanisms also apply to humans. Male sexual differentiation generally depends on a balanced androgen/estrogen ratio. In mice, estrogens impair fetal Leydig cell development, and, as a consequence, testosterone production is decreased.[94] Phthalates that induce male reproductive disorders in rats mainly do so through inhibition of steroidogenesis by the fetal testis,[95,96] but this does not occur *in vitro* with human fetal Leydig cells.[97] Testosterone secretion is responsible for most of the masculinization process, including the development of the male reproductive tract and external genitalia. Therefore, compromised testosterone production may result in hypospadias. In addition, estrogen exposure also suppresses the production of insulin-like factor 3 by fetal Leydig cells.[98] This peptide regulates the growth of the gubernaculum,[99] which is responsible for testicular descent.[100] In humans, a deficiency in androgen production or action seems far more important than estrogen exposure in the etiology of cryptorchidism, since the inhibitory effects of estrogens on testicular steroidogenesis and testicular descent are only mediated through estrogen receptor α in mice,[101] which is not present in the human fetal testes.[102] However, this receptor is expressed and functional in human fetal penile tissue,[103] so a role of estrogen exposure in the induction of hypospadias cannot be excluded. Epidemiologic studies could not confirm this, since prenatal estrogen exposure, including pharmaceutical estrogens, does not seem to be related to hypospadias and cryptorchidism.[104,105]

Alternative mechanisms by which EDCs could cause male reproductive disorders have also been suggested. These mechanisms include disruption of the androgen signaling pathway (e.g., suppression of androgen receptor expression), resistance to the anti-Müllerian hormone (AMH), and inhibition of enzymes involved in the inactivation of sex steroids. However, involvement of these mechanisms in endocrine disruption seems unlikely for various reasons. Although it has been shown that fetal exposure to chemicals that alter the androgen signaling pathway can induce hypospadias and cryptorchidism in rats,[106] the dose needed to induce these effects is very high, which makes this mode for EDC-induced teratogenesis doubtful. AMH is primarily responsible for the regression of the Müllerian tract in male embryos[107] and may play a role in testicular descent.[100] So far, however, no compounds have been identified that affect the production or action of AMH.[93] The same argument can be applied to the inhibition of estrogen sulfotransferases (and probably other enzymes involved in sex steroid metabolism), which increases cellular estradiol bioavailability. Metabolites of various polycyclic aromatic hydrocarbons inhibit this enzyme,[108] but

pharmacological compounds with a similar mechanism of action have not been identified yet

Oxidative stress

In vivo, several drugs, known as redox cycling agents and used in the treatment of, among others, epilepsy, cardiac arrhythmias, and cancer, undergo single electron reduction reactions yielding radical species.[109] In redox cycling reactions which involve oxygen reactive oxygen species (ROS), such as hydrogen oxide, alkyl peroxides, and various radicals (e g , hydroxyl and superoxide), are generated.[110] The creation of ROS is induced by internal and external agents, such as phagocytes, enzymes like cytochrome P450 mono-oxygenases (CYP), irradiation, and exogenous chemicals In much the same manner, the generation of ROS can be decreased or reversed by various enzymes, e g , superoxide dismutase, catalase, and glutathione reductase, and by antioxidants.[111] Endogenous ROS serve as a second messenger in signal transduction[112] and are thought to be important in ion transport, immunological host defense, transcription, and apoptosis of unwanted cells.[113 114] However, ROS can also be harmful by binding covalently or irreversibly to cellular macromolecules Oxidative stress, an imbalance between ROS generation and antioxidant defense mechanisms of a cell or tissue, causes irreversible oxidation of DNA, proteins, and lipids, leading to inactivation of many enzymes and cell death (Figure 2 2).[115] In addition to damaging cellular macromolecules, oxidative stress may affect gene expression by interfering with the activity of redox-sensitive transcription factors and signal transduction by oxidizing thiols.[116] During the prenatal period, this may result in birth defects and growth retardation, and in severe cases in *in-utero* death.[112 117 118]

The developing embryo is especially susceptible to high levels of ROS because of its weak antioxidant defense, in particular in the early stages of organogenesis,[119] although placental enzymes play a role in protecting the fetus against oxidative stress.[120] Oxidative stress is postulated to be involved in the pathogenesis of a wide spectrum of birth defects, including skeletal malformations,[116 121] limb defects,[122 123] neural tube defects,[124 125] cleft lip/palate,[122 126] and cardiovascular defects.[122] Several drugs are known to induce oxidative stress, which is suspected to be their main teratogenic mechanism Among these drugs are thalidomide,[127] phenytoin,[126 128] valproic acid,[129] class III antiarrhythmic drugs,[122 130] iron supplements,[131] and various chemotherapeutic drugs.[111]
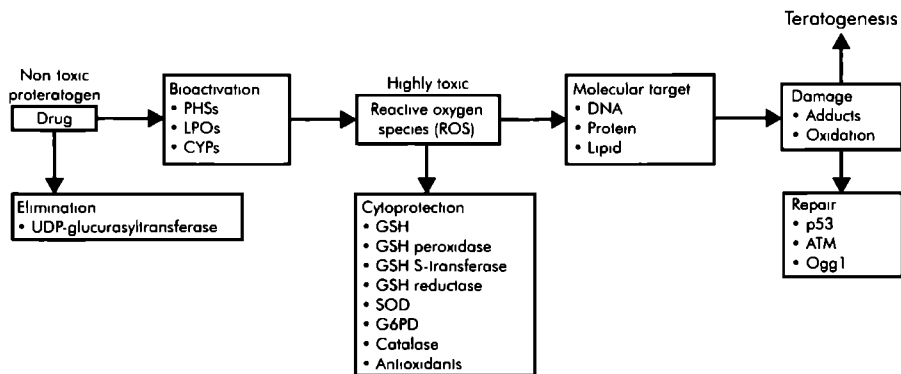
Figure 2.2 Molecular and biochemical determinants of oxidative stress teratogenesis. ATM, ataxia telangiectasia mutated; CYP, cytochrome P450, G6PD, glucose-6-phosphate dehydrogenase, GSH, glutathione, LPO, lipoxygenase; Ogg1, oxoguanine glycosylase; PHS, prostaglandin H synthase; SOD, superoxide dismutase; UDP, uridine diphosphate Modified from Winn and Wells.[115]

However, it is important to notice that ROS are intermediary compounds with unpaired electrons and, as a consequence, have a very short lifetime ranging from nanoseconds to milliseconds. Therefore, ROS are generally too unstable to be transferred from the mother to the developing embryo or fetus. Whenever ROS are increased in embryos, it is the result of embryonic metabolic changes rather than exposure to ROS of maternal origin.[132] Increases in embryonic ROS may be caused by increased enzymatic bioactivation of proteratogens, including bioactivation of the aforementioned drugs. However, most isoforms of the CYP family, which catalyze the bioactivation of many compounds after birth, are expressed at relatively low levels during the embryonic period. Only some isoforms are expressed at levels that could be significant in teratogenesis.[133 134] In contrast, the prostaglandin H synthases (PHSs) have a relatively high expression during the embryonic and fetal period compared with expression after birth.[135,136] The peroxidase component of this enzyme can bioactivate exogenous substances, including phenytoin and related teratogens,[137] to toxic reactive intermediates that initiate ROS formation.[138] There is evidence that lipoxygenases (LPOs), which oxidize proteratogens yielding free radical intermediates, are substantially expressed in embryonic tissues as well.[139] As a result, it is assumed that bioactivation of proteratogens by embryonic PHSs and LPOs is necessary for the formation of ROS and subsequent macromolecule damage in the developing embryo.[118] Additionally, embryonic ROS formation and subsequent oxidative stress may be induced by hypoxia. It is well known from adult cases of cardiovascular diseases[140] that ROS are extensively formed during reperfusion of ischemic tissues, while there is considerable evidence that hypoxia followed by reperfusion is teratogenic in animal studies.[122] Besides embryonic ROS generation, maternal

determinants are thought to play an indirect role in ROS-mediated teratogenesis. Embryonic exposure to proteratogens is altered by maternal pathways that eliminate these compounds or their metabolites before they can cross the placenta. Deficiencies in those pathways increase the maternal plasma concentration of proteratogens and therefore the amount that reaches the embryo. Furthermore, maternal production of factors that interfere with embryonic ROS-mediated signal transduction or alter embryonic determinants of oxidative stress may also contribute to the risk of teratogenicity.[141]

Vascular disruption

Vascular disruption defects are structural birth defects resulting from interference with or extrinsic breakdown of an originally normal prenatal development of the arteries, veins, and capillaries (vasculature).[142,143] Traditionally, it has been stressed that a teratogen exerts its influence on the fetus during the first three months of development. Prenatal exposure to agents which can induce vascular disruption, however, can also induce damage later in pregnancy to structures that were initially formed normally. After birth it may be impossible to determine whether a certain structural anomaly, such as a limb defect, is the result of an intrinsically abnormal developmental process, vascular disturbances, or for example amniotic banding.

Vascular disruption refers to disturbances in the blood circulation in the uterine-placental unit, the placental-fetal unit, or the fetus itself. These disturbances include hyperperfusion, hypoperfusion, hypoxia, and obstruction. They may be caused by acute or chronic decreases in uterine blood flow, vascular infections, or an abnormal anatomy in the uterine-placental unit. Factors such as placental insufficiency, amnion rupture, and umbilical cord obstruction may cause failures in the vascular supply in the placental-fetal unit. In the fetus, disruption of newly formed vessels, external compression, embolic events, premature regression of embryonic vessels, occlusion with venous engorgement, and abnormal regulation of vessel formation lead to vascular disruption.[144] Vasoconstriction of maternal and fetal vessels, hypoperfusion, and obstruction may cause a reduced supply of nutrients to the embryonic tissues, which can affect development and growth of embryonic structures or result in tissue loss. The latter may result in a phenotype similar to a primary malformation.[145] Furthermore, these disturbances may create a state of hypoxia, which is involved in the formation of ROS and oxidative stress.[132]

Exposure to vasoactive substances in pregnancy, especially to those with vasoconstrictive effects, have been hypothesized to play a causal role in vascular disruption defects. These teratogens could decrease placental or fetal blood flow or

affect the development of blood vessels, thereby changing the structure and/or anatomy of the vasculature.[143] In epidemiologic studies, vasoactive therapeutic drugs that have reported associations with the vascular disruption defects described below include misoprostol,[146 147] aspirin,[148 149] ergotamine,[150 151] and pseudoephedrine.[149 152] However, all drugs with vasoconstrictive or vasodilating effects may have the potential to cause birth defects due to vascular disruption.

The types of structural anomalies that may be caused by vascular disruption are determined by the timing during gestation, the location and severity of tissue damage, and the possible presence of secondary adhesion of necrotic tissue with adjacent organs or the amnion.[143] During embryogenesis, vascular disruption results in aberrant differentiation and distortion of contiguous tissues, loss of tissue, and incomplete development of structures within the same or a secondary embryonic developmental field. Anomalies resulting from vascular disruption during the fetal period are usually limited to the areas with disturbed blood supply, to which the peripheral vasculature is most susceptible.[144] Therefore, the majority of defects caused by tissue damage through vascular disruption occur in structures supplied by the most peripheral vasculature, such as the distal limbs and the embryonic intestine.[153 154] Birth defects that were attributed to vascular disruption include terminal limb reductions,[155 156] hydranencephaly/porencephaly,[157 158] gastroschisis,[159 160] small intestinal atresia,[161,162] and Poland anomaly.[163 164] However, there are no known experimental models for the complete range of birth defects caused by vascular disruption. The majority of evidence in support of this mechanism comes from case reports with suspected vascular events such as occlusion, emboli, amnion rupture, and twin placental vessel anastomoses.[143]

Specific receptor- or enzyme-mediated teratogenesis

Many medical drugs act on a specific receptor or enzyme in the human body, leading to a particular mechanism of action. Below we describe the possible effects of inhibition or stimulation of some of these specific receptors and enzymes on fetal development.

Angiotensin-converting enzyme and angiotensin II receptors

The renin-angiotensin system (Figure 2.3) is generally described as a hormonal system that plays an important role in the regulation of blood pressure and in the homeostasis of extracellular fluid volume. The main effector hormone of this system is angiotensin II (AT II), which elevates blood pressure by acting directly on vascular smooth muscle cells to cause vasoconstriction. The components of the renin-angiotensin system are present in the human fetus, although their distribution varies
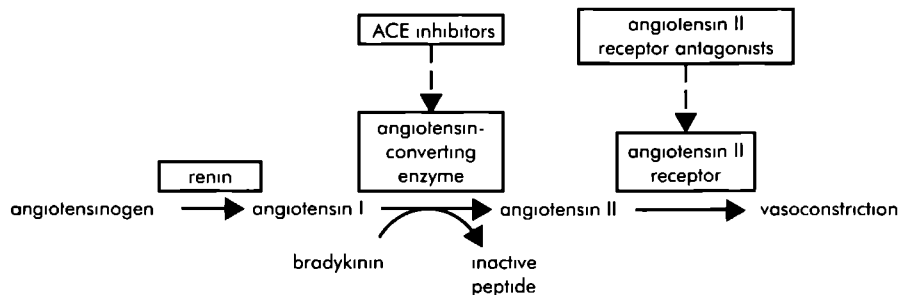
Figure 2.3 The renin-angiotensin system. ACE, angiotensin-converting enzyme

compared to that in adults.[165] Two types of commonly used antihypertensive drugs, the angiotensin-converting enzyme (ACE) inhibitors and the AT II receptor antagonists, may disrupt the fetal renin-angiotensin system and thereby impair fetal development. In contrast to other antihypertensive drugs, ACE inhibitors and AT II receptor antagonists also influence renal function.[166] Therefore, their effects are not exclusively produced through fetal hypotension and vascular disruption. The decrease in fetal renal vascular tone may contribute to a human malformation syndrome that is typical for exposure to ACE inhibitors during the second and third trimesters of pregnancy, characterized by renal tubular dysgenesis and oligohydramnios, their sequelae, including limb contractures and pulmonary hypoplasia, and hypocalvaria.[167 168] Although the two AT II receptor subtypes, AT_1 and AT_2, are expressed in early development,[165] the developmental effects of ACE inhibitors during the first trimester are controversial. However, a recent study showed an increased risk of cardiovascular and central nervous system malformations.[169] The effects of the less often studied AT II receptor inhibitors are considered to be similar to those of ACE inhibitors.

Hydroxymethylglutaryl-coenzyme A reductase

The mevalonate pathway is a complex pathway with cholesterol as an essential product. In embryonic tissues, cholesterol is needed for normal growth patterns, signaling domains in plasma membranes, synthesis of steroid hormones, and activation of Hedgehog morphogens.[170,171] Since Hedgehog proteins act as key regulators of embryonic growth, patterning, and morphogenesis of many structures, down-regulation of the synthesis of these proteins may lead to birth defects.[172 173] Statins inhibit hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway which converts HMG-CoA to mevalonic acid. Therefore, inhibition of this pathway by statins may lead to a wide range of defects. However, epidemiologic studies with appropriate control populations to confirm a statin syndrome in humans have not been performed yet due to the low

frequency of statin use among pregnant women. Although a recurrent pattern of structural defects has been described,[174] a recent study could not confirm this hypothesized pattern.[175]

Histone deacetylase
Histone deacetylases (HDACs) are present in most organisms, in which their best known function is the deacetylation of histones. These are crucial in a number of cellular functions, including the regulation of gene expression by chromatin remodelling. HDACs deacetylate lysine residues on histone tails and condensate chromatin, resulting in limited access of transcriptional activators to the DNA.[176] Therefore, inhibition of HDACs may result in interruption of cell proliferation, differentiation, and apoptosis,[177] which has been shown in cultured tumor cells.[178 179] Although normal cells seem to be relatively resistant to HDAC inhibitors,[180 181] HDAC activity is crucial for embryonic development as is shown by the HDAC1 knockout mice, which die early in development due to growth retardation and proliferation defects.[182] Not much has been published on the effects of HDAC inhibition in the pathogenesis of human birth defects, but animal studies show that it might lead to axial skeletal malformations[183 184] and neural tube defects.[185] Drugs that inhibit HDACs include valproic acid,[186 187] trichostatin A,[188] and salicylates.[189] Furthermore, boric acid, an inactive ingredient used in pharmaceutical preparations and as an antibacterial product in non-prescription products, may induce hyperacetylation in somites.[184]

Cyclooxygenase-1
Non-steroidal anti-inflammatory drugs (NSAIDs) are used for their analgesic, antipyretic, and anti-inflammatory effects induced by acting as an inhibitor of cyclooxygenases (COXs), which catalyze the conversion of arachidonic acid to prostaglandins. Two distinct isoforms have been identified, COX-1 and COX-2. The constitutive form, COX-1, is expressed in most tissues, where it produces prostaglandins that are necessary for various physiologic processes, such as blood pressure regulation and platelet aggregation. COX-2 expression, on the other hand, is induced by inflammatory mediators, producing prostaglandins which are important in inflammation.[190] The anti-inflammatory properties of NSAIDs are due to the inhibition of COX-2, whereas the adverse effects of non-selective NSAIDs, which inhibit both COX isoforms, are the result of COX-1 inhibition.[190] COX-1 inhibition may be involved in the induction of cardiac, midline, and diaphragm defects by non-selective NSAIDs, since these defects were associated with exposure to drugs with a relatively high COX-1/COX-2 ratio in rats and rabbits.[191] Furthermore, COX-2 is not expressed during embryogenesis in rats,[192 193] which strongly suggests that COX-2 does not play a role in NSAID-induced teratogenicity noted in this species.

Acetylsalicylic acid (aspirin), the only NSAID that irreversibly inhibits COX by acetylation,[190] seems to be associated with a higher incidence of malformations than other NSAIDs in animal studies [194] Initially, first trimester exposure to NSAIDs did not seem to be associated with birth defects in humans,[195 196] but recent epidemiologic studies indicate an increased risk of orofacial clefts and cardiovascular defects, especially cardiac septal defects [197 199]

N-methyl-D-aspartate receptors

In the developing brain, N-methyl-D-aspartate (NMDA) receptors appear to play an important role in neuronal migration and in the formation and elimination of synapses [200] Blockade of the NMDA receptor in studies using NMDA receptor antagonists or knock-out mice affect neuronal development,[200 201] which may result in structural abnormalities of the brain due to errors in migration of neuronal and glial elements [202] Rats are most vulnerable to the effects of NMDA receptor antagonists in the first week after birth,[203] during which the expression of NMDA receptors peaks[204] and the brain growth spurt occurs [205] Since the expression of NMDA in humans peaks in week 20-22 of gestation,[206] during which the brain growth spurt starts, and continues throughout the third trimester and postnatally,[207] it has been hypothesized that humans might be susceptible to the effects of NMDA receptor antagonists from 20 weeks of gestation onward [203] Therefore, it may be concluded that exposure to NMDA receptor antagonists, such as amantadine,[208] dextromethorphan,[209] and ketamine,[210] could result in minor malformations of the brain Controversial is the suggested role of NMDA receptor antagonists in the induction of neural tube and neural crest defects, as shown by Andaloro et al [211] using chick embryos These results could not be replicated in mice[36] and the widely used drug dextromethorphan does not seem to be associated with congenital defects in humans [212] Although NMDA receptors are being expressed in the human spinal cord during the first trimester,[213] inhibition of these receptors does not appear to play a role in the induction of neural tube and neural crest defects Therefore, it is questionable whether this mechanism produces major structural birth defects in humans

5-Hydroxytryptamine receptors and transporters

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter, which is derived from the maternal circulation and transported towards the embryo [214] It is involved in a wide range of processes during development, including morphogenesis of craniofacial structures,[215] cranial neural crest migration,[216] and cell proliferation [217] The effects of 5-HT appear to be mediated by 5-HT receptors,[218] G-protein-linked transmembrane receptors with the exception of the 5-HT$_3$ receptor, which is a ligand-gated ion channel At least some of the 5-HT receptor subtypes are

expressed in mice embryos, and these are shown to be involved in the morphogenesis of various embryonic tissues [219 220] Therefore, increased stimulation or suppression of 5-HT receptors by agonists and antagonists may cause birth defects Drugs known to be agonists of some 5-HT receptor subtypes include sumatriptan[221] and buspirone,[222] whereas, among others, risperidone,[223] granisetron,[224] and quetiapine[225] antagonize some 5-HT receptor subtypes Furthermore, the actions of 5-HT are terminated by the uptake of the neurotransmitter by serotonin transporters, implying that prenatal exposure to selective serotonin-reuptake inhibitors (SSRIs) may also cause birth defects This class of antidepressants, which includes fluoxetine, paroxetine, and sertraline, has been shown to cause craniofacial malformations in mice [226] 5-HT also seems to be involved in cardiac morphogenesis,[227 228] indicating that blockade of 5-HT uptake might produce cardiovascular malformations as well In humans, however, the risk of birth defects associated with SSRIs as a group appears to be small,[229 231] although recent reports suggest an association between paroxetine use and birth defects,[232 233] but this has been refuted by others [234] An association between first trimester exposure to fluoxetine and cardiovascular anomalies has been suggested as well [235] Therefore, it may be hypothesized that individual SSRIs may have different effects on the developing embryo Due to the inconsistencies in the results of epidemiologic studies, one may suspect that other issues also play a role in this possible association, including disease status of the mother and other confounding factors, such as detection bias and use of concomitant medications

γ-Aminobutyric acid receptors

In vertebrates, γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter, which binds to specific transmembrane GABA receptors Extraneuronal GABA-ergic systems are thought to be present in other tissues as well, including the testis,[236] oviduct and ovary,[236 237] and pancreas,[238] where GABA is hypothesized to play a morphogenetic role during embryonic development [239] The extraneuronal GABA-ergic system also seems to play an important role in the normal development of the palate,[240] but the exact function of this system in non-neural tissues is still unknown The major group of drugs that exert their pharmacologic actions through GABA receptors are benzodiazepines, which enhance the effects of GABA [241] Although these drugs are commonly used during pregnancy and neonatal complications, such as the 'floppy infant syndrome' and the 'withdrawal syndrome', have frequently been observed, data on the teratogenicity of benzodiazepines are scarce and inconsistent In some epidemiologic studies, use of benzodiazepines in the first trimester has been associated with orofacial clefts,[242] cardiovascular malformations,[243] and gastrointestinal tract atresia,[244] but other studies did not find an association with birth defects [245 247]

Carbonic anhydrase

Carbonic anhydrases are metalloenzymes that catalyze the reversible hydration of CO_2 into the bicarbonate ion and protons. This reaction is involved in many biological processes, including pH homeostasis, respiration, biosynthetic reactions, and bone resorption.[248 249] Several cytoplasmic and membrane-bound carbonic anhydrase isoenzymes are expressed in various tissues in developing human and mouse embryos,[250 252] and inhibitors of carbonic anhydrase, such as acetazolamide, which is used in the treatment of epilepsy, altitude sickness, edema, and sleep apnea, have been associated with birth defects, especially limb deformities.[253 254] A reduction in embryonic intracellular pH is thought to be the teratogenic mechanism of carbonic anhydrase inhibitors.[254] Intracellular pH has been shown to control or to be associated with various cellular functions, including protein synthesis, proliferation, and glycolysis.[255] Interference with these processes may result in abnormal development, but evidence of the existence of this mechanism in humans is lacking.

Summary

From the literature, we identified six principal teratogenic mechanisms associated with medical drug use. Besides the fact that almost all medical drugs classified by Schwarz *et al.*[12] as U.S. FDA class X are associated with at least one of these mechanisms, various other prescription and over-the-counter drugs may produce teratogenic effects through these mechanisms. Increased risks for specific birth defects have been observed for some medical drugs after use in human pregnancy, which strengthens the evidence in favor of the associated teratogenic mechanisms. However, since the possibilities to conduct experiments during human pregnancy are very limited, the major part of the evidence in support of various mechanisms described above was derived from animal studies, in which the dosages administered were often far above the therapeutic dosage schedules used in humans. Therefore, we cannot be sure that these mechanisms also apply to humans. In addition, some mechanisms share similar pathways and some drugs may be involved in multiple mechanisms, e.g., valproic acid. Nevertheless, the identification of teratogenic mechanisms are critical for research purposes, in particular for observational studies, in which specific medications with a similar teratogenic mechanism might be combined to increase study power. It may have implications for drug development and for prescribing multiple drugs to women of reproductive age as well, especially since combinations of seemingly unrelated drugs may produce specific teratogenic mechanisms, which may strongly increase the risk of birth defects. Given that discontinuing a certain medication may pose even a higher risk for severe complications than continuing with the use of a possible teratogen, the benefits for

the mother should always be balanced against the risks for the (unborn) child when prescribing drug treatment to pregnant women.

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Chapter 3

Drugs associated with teratogenic mechanisms: prescription rates among pregnant women and a systematic review of effects

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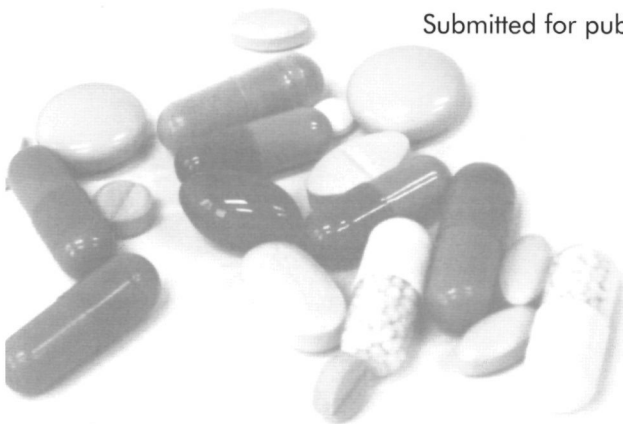
Abstract

Background

The main teratogenic mechanisms of medical drugs were previously described, but insight in the use of prescription medication associated with these mechanisms and knowledge on the human teratogenic effects of these drugs are lacking. Therefore, we determined prescription rates of drugs associated with teratogenic mechanisms and evaluated the current knowledge on human teratogenic risks of these drugs.

Methods

We estimated prescription rates of the medical drugs involved among Dutch women who gave birth between 1998 and 2007 using data from the IADB.nl database. In addition, time trends in first trimester prescription rates were evaluated. Furthermore, we conducted a systematic review of the literature to study the human teratogenic effects of these medical drugs. Of 13,771 potential articles, 247 were included in the systematic review.

Results

In 177 per 1,000 pregnancies (95% confidence interval 172-182) in our study population, at least one drug associated with a teratogenic mechanism was dispensed in the first trimester. The prescription rates increased over time for vasoactive drugs, selective serotonin-reuptake inhibitors (SSRIs), and serotonin receptor agonists/antagonists. Epidemiologic studies assessing the teratogenic risks were identified for less than half of the drugs included in this study. For a number of drugs, including acetaminophen, aspirin, antihypertensive medication, carbamazepine, phenobarbital, valproic acid, clomiphene, and some SSRIs, in particular fluoxetine and paroxetine, associations between exposure in early pregnancy and specific birth defects were observed in both cohort and case-control studies. However, for most drugs the numbers of exposed infants were too small to draw any conclusions regarding their human teratogenic risks.

Conclusions

Although frequently prescribed in the first trimester of pregnancy, evidence for the presence or absence of human risks of birth defects is scarce or non-existent for the majority of medical drugs associated with teratogenic mechanisms.

Background

In developed countries, prescription drug use is common during pregnancy with prevalence estimates ranging from 27% to 99%, depending on the data sources used and the types of medication included.[1] However, the human teratogenic risks are unknown for more than 90% of drug treatments approved for marketing in the United States since 1980.[2] One of the reasons is that pregnant women are often excluded from participation in pre-marketing clinical trials. In addition, results obtained from animal studies are not always predictive for a teratogenic effect in humans. Nevertheless, medication use is occasionally unavoidable in the treatment of women of reproductive age and during pregnancy, for instance among women with epilepsy, diabetes, or severe hypertension.

Several teratogenic and non-teratogenic factors play a role in the etiology of birth defects. A particular birth defect may be caused by many different factors, such as environmental exposures, physical conditions, medication, and genetic defects. Alternatively, specific pathogenetic processes may result in various outcomes for one chemical exposure depending on factors, such as genetic susceptibility, embryonic age, and dose and duration of exposure.[3,4] The most important teratogenic mechanisms inducing birth defects associated with medical drug use were recently described,[5] but it is unknown how many pregnant women are actually exposed to drugs grouped within a specific teratogenic mechanism. As seemingly unrelated medications may exhibit similar teratogenic mechanisms, which, in turn, may increase the risk of specific birth defects even more when combined, serious implications for prescribing behavior could be postulated. And this issue not only pertains to pregnant women, but to all women of reproductive age since a significant proportion of pregnancies is unintended.[6]

Previous studies have mainly reported on the overall prevalence of prescription drug use among pregnant women or focused on drugs within particular pregnancy risk classification classes, certain anatomic or pharmacological/therapeutic subgroups, or specific types of medications. Therefore, we determined prescription rates of drugs associated with the teratogenic mechanisms described previously among pregnant Dutch women. Furthermore, we conducted a systematic review to provide more insight in the human teratogenic risks of these drugs.

Methods

Prescription rates among pregnant women

This study was performed using data collected in the IADB nl database, a longitudinal population-based pharmacy database that contains information on prescriptions dispensed from community pharmacies in the Netherlands The methods of the IADB nl database have been described in detail elsewhere [7 8] In short, the IADB nl database includes all pharmacy prescriptions for an estimated population of 220,000 people from 1994 to 1999 and was expanded to a population of approximately 500,000 since 1999 Registration within the IADB nl database is irrespective of health insurance and is considered representative for the general population Each prescription record has information on the name of the drug, which is coded according to the Anatomical Therapeutic Chemical (ATC) classification,[9] the date of dispensing, the amount dispensed, dose regimen, and the prescribing physician The indication for the prescription is unknown Although the data are anonymous, each patient has a unique identifier and date of birth, sex, and address code of the patients are known In the Netherlands, the medication records for each patient are virtually complete as almost everyone is registered with a single pharmacy and all pharmacies use computerized dispensing records [10] The IADB nl database does not include information on over-the-counter drugs and medication dispensed during hospitalizations

To identify mothers, data from the 'Pregnancy IADB' were used, which were extracted from the main IADB nl database For each infant, the female person 15-50 years older than the infant with the same address code was considered to be the mother, provided that there were no other women in that age group registered at the same address code Using this method, of which the validity is described by Schirm *et al*,[11] approximately 65% of the mothers could be identified As only the infant's date of birth is known, the theoretical conception date was determined as the date of birth minus 273 days (i e 9 months) Between January 1, 1998, and December 31, 2007, 27,040 pregnancies were identified Each pregnancy was subdivided into three trimesters of 13 weeks In addition, prescriptions dispensed in the three months before pregnancy (trimester 0) were included

The prescription drugs considered to be associated with the teratogenic mechanisms described by van Gelder *et al*[5] are shown in Table 3 1 As we were primarily interested in drugs that act systemically, locally acting drugs were excluded from the analyses Per trimester, we counted the number of pregnancies in which a prescription for the drugs under study was dispensed If a specific drug was prescribed multiple times during one trimester, it was counted only once Additionally, prescriptions covering multiple trimesters were counted only in the trimester in which

Table 3.1 Categorization of medical drugs considered to be associated with specific teratogenic mechanisms,[5] including their ATC codes

Teratogenic mechanism	ATC codes
Folate antagonism	
Anti-epileptics	N03AA02, N03AA03, N03AB02, N03AF01, N03AG01, N03AX09
DHFR inhibitors	A07EC01, C03DB02, C03EA01, C03EA03, J01EA01, L04AX03, N03AX09, P01BD01
Other drugs	A10BA02, A10BD, C10AC01, C10AD02, L04AD01
Neural crest cell disruption	
Retinoids	A11CA01, D05BB, D10AD, D10BA01, D11AX19, L01XX14
Other drugs	B01AC13, B01AC16, B01AC17, C02KX01, C02KX02, C02KX03, J02AB02, L01XX25, L04AA23
Endocrine disruption	
Oral contraceptives	G02BB, G03AA, G03AB, G03F
Drugs used in fertility treatment	G03C, G03D, G03G
Oxidative stress	B01AC06, B03A, C01BD, C02AB01, C02DB02, C08CA05, J01AA, J01XE01, J04AC01, J04AM02, J05AF01, J05AR01, J05AR04, L01A, L01BB02, L01BC02, L01CB01, L01DB02, L01DC03, L01XA01, L01XX05, L04AX03, N02BA01, N02BE01, N03AA02, N03AB02, N03AE01, N03AG01, N04BB01, N05AA, N05AB, N05AC, N05BA, P01AB01, R03AC03, R03CC03, R06AD
Vascular disruption	A02BB01, C01CA03, C01CA24, C02, C03, C07, C08, C09, M01A, N02BA, N02CA52, N02CC, R01AA05, R01AA07, R03A, R03C, R03CA02
ACE inhibitors / AT II receptor antagonists	C09A, C09B, C09C, C09D
HMG-CoA reductase inhibitors	C10AA, C10BA, C10BX
HDAC inhibitors	A11HA01, N02BA, N03AG01, N05AL
COX inhibitors	M01A, N02BA
NMDA receptor antagonists	N02AX02, N02AX52, N04BB01, N06DX01, N07BC02, N07XX02, R05DA09
Serotonin signaling disturbance	
SSRIs	N06AB
Other drugs	A03FA01, A03FA02, A04AA, C02CA06, C03KD01, C07AA02, C07AA03, C07AA05, G02CB03, N02CA, N02CC, N02CX01, N04BC01, N04BC02, N05AA01, N05AD05, N05AE03, N05AG02, N05AH, N05AX08, N05AX12, N05AX13, N05BE01, N06AA02, N06AA04, N06AA09, N06AA10, N06AA12, N06AA21, N06AX03, N06AX05, N06AX11, N06AX22, N06DX01, R06AX02, R06AX17
GABA receptor antagonists	M03BX01, N01AF, N01AG, N03AA, N03AE01, N05BA, N05CA, N05CB
Carbonic anhydrase inhibition	N03AX11, S01EC01

ACE, angiotensin-converting enzyme; AT II, angiotensin II; ATC, Anatomical Therapeutic Chemical classification; COX, cyclooxygenase; DHFR, dihydrofolate reductase, GABA, γ-aminobutyric acid, HDAC, histone deacetylase; HMG-CoA, hydroxymethylglutaryl-coenzyme A, NMDA, N-methyl-D-aspartate, SSRI, selective serotonin-reuptake inhibitor.

they were dispensed. The prescription rate was calculated as the number of pregnancies per 1,000 in which the mother received one or more prescriptions for a drug class or group of drugs associated with a specific teratogenic mechanism within one trimester. We also evaluated two-years time trends in the prescription rates in the

first trimester of pregnancy using the chi-square test for trend in PASW Statistics version 18 0 2 for Windows (SPSS Inc, Chicago, IL)

Systematic review

We also conducted an extensive literature search of MEDLINE (January 1946 through January 2012) and EMBASE (January 1974 through January 2012) using 287 terms for the medical drugs of interest We used MeSH headings for the generic names of the individual drugs and therapeutic classes whenever applicable, focusing on adverse effects [ae] and toxicology [to], in combination with MeSH headings and terms for birth defects in general (congenital abnormalities, congenital disorder, birth defect) and a number of specific birth defects related to the mechanisms studied (neural tube defects, congenital heart defects, cleft lip, cleft palate, congenital limb deformities, hypospadias, gastroschisis) Additional articles were identified from the reference lists of published papers and from three books on teratogenic agents [4 12 13] English-language articles describing the results of human studies in which the outcome was a major birth defect were included Articles were excluded from the systematic review if they did not specify birth defect subtypes or the drug under study (e g , studies on any antidepressant) or if the drug was not used in the etiologically relevant time period for birth defects Results from studies on associations between anti-epileptic drugs and birth defects were only included if monotherapy was used In addition, cohort studies were only included if they prospectively collected information on medication use before the outcome of the pregnancy was known Case-control studies with less than 100 cases were excluded because of power limitations, as well as case-control studies in which self-reported data were collected >2 years after delivery

The initial search identified 13,771 citations A title and abstract review resulted in 535 original research articles, which were reviewed in full Of these, 247 articles were included in the systematic review (Figure 3 1) The studies included were divided in four categories according to their study design population-based cohort studies, cohort studies using data from voluntary pregnancy exposure registries, case-control studies using population controls, and case-control studies using malformed control subjects All infants with malformations were classified according to the 64 standard EUROCAT birth defect subgroups [14] Because of coding and reporting issues (e g , classification dependent on severity of the defect), we excluded hydrocephalus, microcephaly, hypoplastic right heart, patent ductus arteriosus, cystic adenomatous malformations of the lung, renal anomalies, indeterminate sex, clubfoot, hip dislocation, skeletal dysplasias, amniotic bands, and skin disorders Furthermore, known or suspected genetic syndromes, microdeletions, and chromosomal
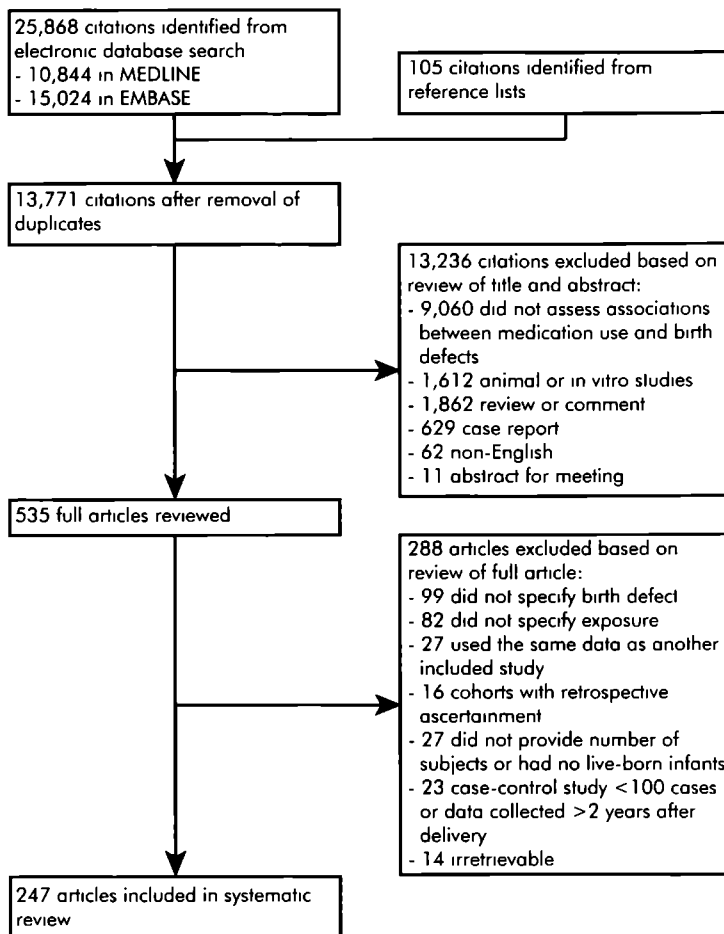
Figure 3.1 Review and selection of articles in the systematic review

abnormalities were excluded as these are very unlikely to result from teratogenic exposures in early pregnancy.

Based on the cohort studies included, the prevalence of each birth defect among live-born infants exposed to a specific drug during development was calculated by dividing the total number of cases from the different studies by the total number of live-born infants exposed to the drug. As hypospadias only occurs in boys, we assumed that the proportion of boys was 0.51 among live-births to calculate the number of exposed infants at risk for this birth defect.[15] For birth defect groups with at least two exposed cases, the prevalence observed was compared with prevalence estimates for the specific defect among live-born infants in Europe and the United

States (Table 3.2)[16 17] using chi-square tests. For the case-control studies, we extracted the total and exposed numbers of case and control subjects (including stillbirths and terminations of pregnancy) and the crude and adjusted odds ratios

Table 3.2 Prevalence estimates for specific birth defects among live-born infants in Europe[16] and in the United States.[17]

Birth defects	Prevalence (per 10,000 live births)	
	Europe (n=6,856,583)	United States (n=1,232,191)
Anencephaly	0.40	3 62
Encephalocele	0.33	1 54
Spina bifida	1.99	5.73
Arhinencephaly/holoprosencephaly	0 31	NR
Anophthalmia/microphthalmia	0.77	NR
Congenital cataract	1 06	2.15
Congenital glaucoma	0.24	NR
Anotia	0.28	NR
Common truncus arteriosus	0.75	0.55
Transposition of great vessels	2.79	2.42
Single ventricle	0.35	0.94
Ventricular septal defect	26.35	36.67
Atrial septal defect	19.48	8.37
Atrioventricular septal defect	1.11	3.74
Tetralogy of Fallot	2 43	4.58
Tricuspid atresia and stenosis	0 53	NR
Ebstein's anomaly	0.32	0 52
Pulmonary valve stenosis	3 15	4.18
Pulmonary valve atresia	0 59	0.47
Aortic valve atresia/stenosis	1.00	1.10
Hypoplastic left heart	1.34	2.51
Coarctation of aorta	2.96	3.88
Total anomalous pulmonary venous return	0.41	NR
Choanal atresia	0.86	1.23
Cleft lip ± cleft palate	7 65	9.55
Cleft palate	4.90	5 45
Esophageal atresia	1 88	2.16
Duodenal atresia or stenosis	0.95	1 62
Small intestinal atresia	0.58	1 63
Anorectal atresia and stenosis	2.22	3 71
Hirschsprung's disease	1.03	1 84
Biliary atresia	0.29	0 80
Annular pancreas	0.13	NR
Diaphragmatic hernia	1 96	2 27
Gastroschisis	1.84	1 98
Omphalocele	1.16	2 73
Bladder exstrophy/epispadias	0.40	0 15
Posterior urethral valve/prune belly	0.52	1 11
Hypospadias[a]	26.06	59 35
Limb reduction	3.84	5 42
Polydactyly	7.23	15 53
Syndactyly	5.03	NR
Craniosynostosis	1.12	3 90
Situs inversus	0 46	NR

NR, not reported.

[a] Based on an estimated proportion of 0.51 of boys among live births

(ORs) with their confidence intervals (CIs). When the studies included did not report the crude OR, the effect estimate was calculated based on the number of subjects. The statistical analyses for the systematic review were performed using Episheet.[18]

Results

Prescription rates among pregnant women

The prescription rates per trimester for drugs associated with teratogenic mechanisms are shown in Table 3.3. In 4,784 out of the 27,040 pregnancies in our study population (177 per 1,000), at least one drug associated with a teratogenic mechanism was dispensed in the first trimester. The total prescription rate was higher in the three months before pregnancy (237 per 1,000) and an increasing pattern was seen in the second and third trimesters with prescription rates of 224 and 344 per 1,000, respectively. This pattern was mainly caused by an increase in the prescription rates for oxidative stress inducers, in particular iron preparations, with prescription rates of 39.6 per 1,000 in the first, 148 per 1,000 in the second, and 273 per 1,000 in the third trimester of pregnancy. In the first trimester, the highest prescription rates were observed for oxidative stress inducers (87 per 1,000), vasoactive drugs (50 per 1,000), endocrine disrupting drugs (38 per 1,000), drugs that influence serotonin signaling (25 per 1,000), and cyclooxygenase (COX) inhibitors (21 per 1,000). Compared to the three months before pregnancy, fewer prescriptions were dispensed for most drug groups, but especially for folate antagonists, oral contraceptives and fertility drugs, vasoactive drugs, COX inhibitors, serotonin signaling disturbers, and γ-aminobutyric acid (GABA) receptor antagonists.

Figure 3.2 shows the prescription rates for the 12 most prescribed drug groups in the first trimester over the study period (1998-2007). Overall, the first trimester prescription rates for any drug associated with teratogenic mechanisms were nearly constant over time (p=0.35; Figure 3.2A). However, when looking at specific drug groups, the prescription rates in the first trimester decreased over time for oxidative stress inducers (p<0.001; Figure 3.2A), iron preparations (p<0.001; Figure 3.2A), and COX inhibitors (p=0.05; Figure 3.2B), whereas an increasing time trend was observed for vasoactive drugs (p=0.02; Figure 3.2A), selective serotonin-reuptake inhibitors (SSRIs; p<0.001; Figure 3.2C), and serotonin receptor agonists/antagonists (p<0.001; Figure 3.2C). We did not observe time trends in the prescription rates in the first trimester for endocrine disrupting drugs in general, nor for oral contraceptives or fertility drugs in particular, for folate antagonists, and for GABA receptor antagonists.

Table 3.3 Prescription rates of medications considered to be associated with teratogenic mechanisms among pregnant women in the IADB nl database 1998-2007 (*n*=27,040).

Drug group	Prescription rate per 1,000 pregnancies (95% confidence interval)			
	Trimester 0	Trimester 1	Trimester 2	Trimester 3
Any drug associated with a teratogenic mechanism	236 6 (231.5-241.6)	176.9 (172 4-181 5)	224 4 (219 5-229 4)	344 2 (338.5-349.8)
Folate antagonists	18 0 (16 4-19 6)	11.0 (9.7-12.2)	8 1 (7 0-9 2)	11.2 (10.0-12.5)
Anti-epileptics	2 5 (1 9-3 1)	2.3 (1 7-2 9)	2 6 (2 0-3.2)	2 7 (2 0-3.3)
DHFR inhibitors	14.9 (13.5-16.4)	8 2 (7 2-9 3)	5.3 (4.5-6 2)	7 8 (6 8-8 9)
Neural crest cell disruptors	0.8 (0.4-1 1)	0 4 (0.1-0.6)	0 1 (0.0-0 2)	<0 1 (0.0-0.1)
Retinoids	0.6 (0 4-1.0)	0.3 (0 1-0 6)	0 1 (0.0-0.2)	<0 1 (0 0-0.1)
Other neural crest cell disruptors	0.1 (0 0-0 2)	<0.1 (0.0-0.1)	0 0	0.0
Endocrine disrupting drugs	92.6 (89.1-96.0)	37 5 (35 3-39 8)	4 6 (3.8-5.4)	5 3 (4 4-6 1)
Oral contraceptives	59 8 (57 0-62 7)	13 6 (12.2-15 0)	3 3 (2.6-4 0)	3.8 (3.1-4.6)
Drugs used in fertility treatment	39.5 (37.2-41 9)	24 7 (22 8-26.5)	1.4 (0.9-1 8)	1 5 (1 1-2 0)
Oxidative stress inducers	64 4 (61 5-67 3)	87 3 (83.9-90.6)	188 4 (183 7-193 0)	310.7 (305 2-316 2)
Iron preparations	10 1 (8 9-11 3)	39 6 (37.3-41.9)	147.5 (143 3-151.7)	273 0 (267.7-278.3)
Vasoactive drugs	90 2 (86 7-93.6)	49.6 (47 0-52.2)	33.2 (31.1-35.4)	42 6 (40 2-45 0)
Antihypertensive medication	8.7 (7.6-9 8)	7.6 (6 5-8 6)	8.4 (7 3-9.5)	18 1 (16.5-19 7)
ACE inhibitors/AT II receptor antagonists	0 6 (0 3-0 9)	0.4 (0 2-0.7)	0 1 (0 0-0.3)	0 3 (0 1-0.5)
HMG-CoA reductase inhibitors	0.7 (0 4-1 1)	0.3 (0 1-0 5)	0 1 (0 0-0.3)	0.3 (0.1-0.5)
HDAC inhibitors	3.6 (2.8-4.3)	2 0 (1 4-2 5)	1.2 (0.8-1.6)	1.4 (1 0-1 9)
COX inhibitors	58.8 (56.0-61 6)	21 4 (19.7-23.1)	5 5 (4 6-6 4)	4.9 (4 1-5.7)
NMDA receptor antagonists	1.6 (1.1-2.1)	0 6 (0 3-0 8)	0 3 (0.1-0 6)	0 4 (0 1-0 6)
Drugs that influence serotonin signaling	33 0 (30.9-35 1)	25 2 (23.4-27.1)	12 1 (10 8-13.4)	11.8 (10 5-13 1)
SSRIs	15 3 (13.9-16 8)	10 9 (9 7-12 2)	6.7 (5.7-7 7)	6 5 (5.6-7 5)
Serotonin receptor agonists or antagonists	19 0 (17 4-20 7)	15.0 (13.6-16.5)	5 7 (4.8-6.6)	5 4 (4 5-6 3)
GABA receptor antagonists	17 6 (16 1-19.2)	10 1 (8.9-11 2)	6.0 (5.1-7 0)	7.2 (6 2-8 3)
Carbonic anhydrase inhibitors	<0.1 (0 0-0 1)	0 0	0.1 (0.0-0 2)	0.1 (0 0-0 2)

ACE, angiotensin-converting enzyme; AT II, angiotensin II, COX, cyclooxygenase, DHFR, dihydrofolate reductase, GABA, γ-aminobutyric acid, HDAC, histone deacetylase, HMG-CoA, hydroxymethylglutaryl-coenzyme A, NMDA, *N*-methyl-D-aspartate, SSRI, selective serotonin-reuptake inhibitor.
Trimester 0 represents the three months before pregnancy, trimester 1-3 is the pregnancy period
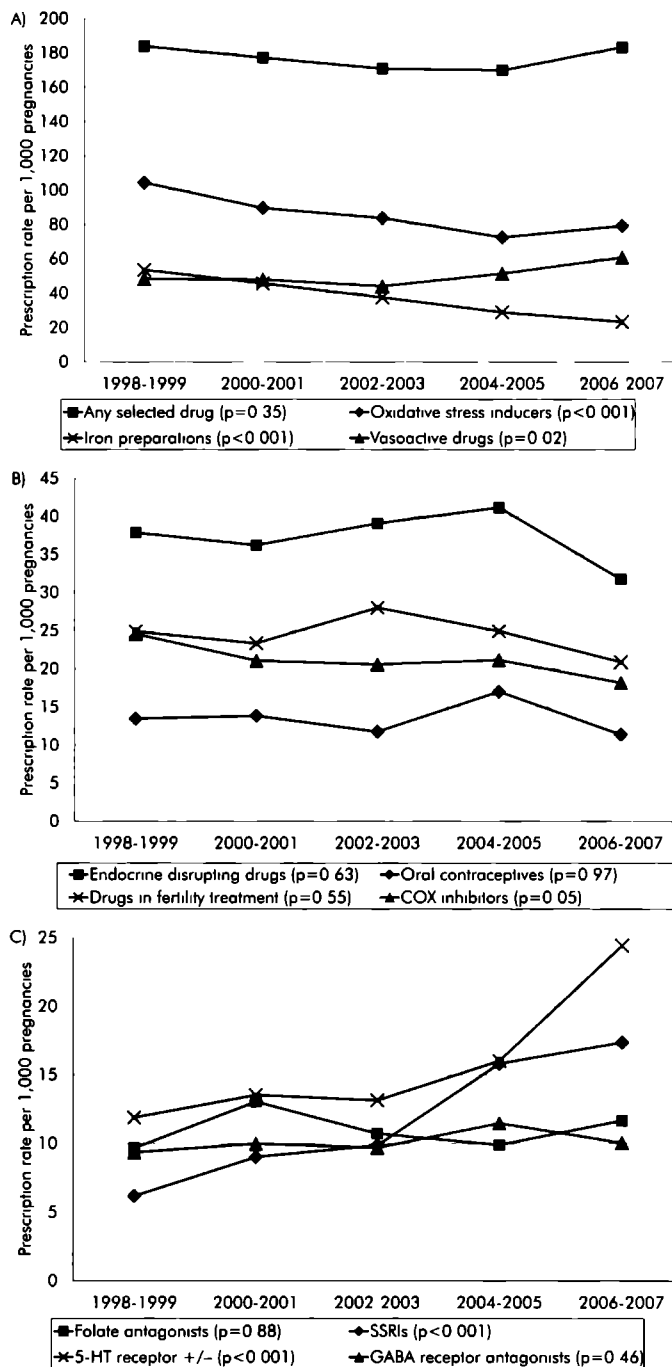
Figure 3.2 First trimester prescription rates per 1,000 pregnancies for the most commonly dispensed drug groups over the study period (1998-2007) The p-values indicate whether a time trend was observed

Systematic review

A total of 247 studies met our inclusion criteria for the systematic review (Figure 3.1). Of these, 115 reported population-based cohort studies, 62 reported the results of cohort studies using data from voluntary reporting systems, 57 reported case-control studies with population controls, and 18 reported case-control studies with malformed controls. A total of 5 case-control studies used both population and malformed control groups and were included in both categories. The results for a total of 101 and 57 drugs or drug groups were included from cohort studies and case-control studies, respectively. The list of included studies is provided in Appendix 3.1.

Table 3.4 shows the numbers of cohort studies and exposed infants that contributed to the prevalence estimates for the individual drugs. The drugs with the largest number of infants studied in relation to birth defects in population-based cohorts were acetaminophen (26,479 exposed infants from 2 studies), SSRIs (15,287 exposed infants from 5 studies), aspirin (16,091 exposed infants from 2 studies), non-steroidal anti-inflammatory drugs (NSAIDs; 5,560 exposed infants from 2 studies), and progesterone (4,306 exposed infants from 8 studies). However, the 4 drugs studied most often were all anti-epileptic drugs: phenytoin (13 studies with 527 exposed infants), carbamazepine (12 studies with 1,370 exposed infants), phenobarbital (10 studies with 1,815 exposed infants), and valproic acid (10 studies with 492 exposed infants). In the cohorts from voluntary reporting systems, most infants were exposed to lamotrigine (3,518 infants from 4 studies), carbamazepine (1,528 infants from 4 studies), valproic acid (1,345 infants from 5 studies), and fluoxetine (643 infants from 5 studies). In 4 studies, isotretinoin was the exposure of interest, but these studies together contained only 60 live-born infants. For many other drugs or drug groups, very small numbers of infants were included in cohort studies.

The numbers of case-control studies and cases included for specific drugs are given in Table 3.5. For 5 drugs or drug groups, exposure was assessed in more than 10,000 cases with population controls: clomiphene (21,024 cases from 5 studies), oral contraceptives (16,149 cases from 9 studies), acetaminophen (14,153 cases from 5 studies), SSRIs (11,171 cases from 2 studies), and promethazine (11,130 cases from 2 studies). The teratogenic potential of aspirin and antihypertensive medication was investigated in 8 (8,351 cases) and 4 (6,858 cases) studies, respectively. The other drugs and drug groups were included in less than 4 case-control studies with population controls, whereas case-control studies with malformed controls were only rarely conducted. The drugs most often assessed in these studies

Table 3.4 Summary of the cohort studies included in the systematic review.

Drug	Mechanism	Population-based cohorts		Cohorts from voluntary reporting systems	
		No. of studies	No. of exp. infants	No. of studies	No. of exp. infants
5-Fluorouracil	OS	1	4	0	0
Acetaminophen	OS	2	26,479	2	66
Acetazolamide	CA	0	0	1	1
Amiodarone	OS	2	6	0	0
Amitriptyline	5-HT	0	0	1	89
Antihypertensive medication	VD	1	1,430	1	400
ACEi/AT II blocker	VD+AT	0	0	1	188
ACE inhibitor	VD+AT	2	268	1	8
Captopril	VD+AT	1	9	1	15
Enalapril	VD+AT	1	9	1	6
Lisinopril	VD+AT	1	9	0	0
AT II blocker	VD+AT	3	30	1	30
Acebutolol	VD	1	6	0	0
Atenolol	VD	1	5	0	0
Calcium-channel blocker	VD	1	25	2	293
Amlodipine	VD	0	0	1	31
Diltiazem	VD	0	0	1	29
Felodipine	VD	1	3	0	0
Nifedipine	OS+VD	0	0	1	51
Verapamil	VD	0	0	1	55
Pindolol	VD	1	7	0	0
Sotalol	VD	1	1	0	0
Benzodiazepines	OS+GA	2	1,391ª	2	459
Alprazolam	OS+GA	1	6	1	276
Chlordiazepoxide	OS+GA	1	18	0	0
Clobazam	OS+GA	0	0	1	9
Clonazepam	OS+GA	4	46	1	9
Diazepam	OS+GA	3	39	0	0
Lorazepam	OS+GA	1	1	0	0
Bromocriptine	5-HT	0	0	1	375
Cabergoline	5-HT	2	173	1	49
Carbamazepine	5-HT	12	1,370ª	4	1,528ª
Chlorpromazine	OS	1	57	0	0
Cisapride	5-HT	0	0	1	88
Clomipramine	5-HT	1	1,029ª	1	87
Cyclosporine	FA	2	63	0	0
Doxepin	5-HT	0	0	1	8
Ergotamine	VD	1	213	0	0
Imipramine	5-HT	0	0	1	27
Isoniazid	OS	1	17	0	0
Isotretinoin	NC	0	0	4	60ª
Lamotrigine	FA	2	135	4	3,518ª
Maprotiline	5-HT	0	0	1	77
Mercaptopurine	OS	2	11	0	0
Metformin	FA	3	327	0	0
Methotrexate	FA+OS	2	6	1	19
Metoclopramide	5-HT	1	189	1	158
Metronidazole	OS	3	102	1	131
Mianserin	5-HT	1	63	1	37
Mirtazapine	5-HT	1	154	2	75
Misoprostol	VD	1	118	1	67
Naratriptan	VD+5-HT	0	0	1	46
Nitrofurantoin	OS	1	32	0	0
NSAIDs	VD+CI	2	5,560ª	0	0

Table 3.4 (Continued)

Drug	Mechanism	Population-based cohorts		Cohorts from voluntary reporting systems	
		No. of studies	No. of exp. infants	No. of studies	No. of exp. infants
Diclofenac	VD+CI	0	0	1	123
Ibuprofen	VD+CI	0	0	1	22
Tiaprofenic acid	VD+CI	0	0	1	7
Nortriptyline	5-HT	0	0	1	4
Olanzapine	5-HT	0	0	1	18
Ondansetron	5-HT	0	0	1	169
Oxomemazine	OS	1	14	0	0
Phenobarbital	FA+OS+GA	10	1,815[a]	2	294[a]
Phenytoin	FA+OS	13	527[a]	1	17
Pizotifen	5-HT	1	12	0	0
Primidone	FA+GA	4	86[a]	0	0
Promethazine	OS	2	2,775[a]	1	13
Pyrimethamine	FA	0	0	1	149
Rizatriptan	VD+5-HT	0	0	1	23
Salbutamol	VD	1	648[a]	0	0
Salicylates	HI	1	146	0	0
Aspirin	OS+HI+CI	2	16,091[a]	0	0
Sex hormones	ED	2	1,235	0	0
17OHP	ED	1	140	0	0
Allylestrenol	ED	1	27	0	0
Clomiphene	ED	7	1,966	0	0
Diethylstilbestrol	ED	2	1,053[a]	0	0
hCG	ED	2	345	0	0
Horm. pregn. test	ED	1	661	0	0
Oral contraceptives	ED	3	1,308[a]	1	99
DMPA	ED	1	15	0	0
Levonorgestrel	ED	1	272	0	0
Progesteron	ED	8	4,306[a]	0	0
Stilbestrol	ED	1	2	0	0
SSRIs	5-HT	5	15,287[a]	2	353
Citalopram	5-HT	3	472[a]	1	184
Escitalopram	5-HT	1	7[a]	1	21
Fluoxetine	5-HT	2	1,653[a]	5	643
Fluvoxamine	5-HT	0	0	2	102
Paroxetine	5-HT	4	2,881[a]	3	499
Sertraline	5-HT	3	3,488[a]	1	61
Statins	HMG	1	61	3	264
Sulfasalazine	FA	1	40	0	0
Sumatriptan	VD+5-HT	2	725	2	544
Tetracycline	OS	1	341[a]	0	0
Thalidomide	OS	1	5	0	0
Topiramate	CA	1	7	2	103
Trazodone	5-HT	0	0	2	27
Tretinoin	NC	1	212	2	177
Valproic acid	FA+OS+HI	10	492[a]	5	1,345[a]
Vitamin A	NC	0	0	1	311
Zidovudine	OS	2	358[a]	2	58

5-HT, influence serotonin signaling; 17OHP, 17-hydroxyprogesterone; ACE, angiotensin-converting enzyme, AT, disturb angiotensin-renin system, AT II, angiotensin II, CA, carbonic anhydrase inhibition, DMPA, depot medroxyprogesterone acetate; FA, folate antagonism, GA, GABA receptor antagonist; hCG, human chorionic gonadotropin; HI, HDAC inhibition; HMG, HMG-CoA reductase inhibition; horm pregn test, hormonal pregnancy test; NC, neural crest cell disruption; NSAID, non-steroidal anti-inflammatory drug, OS, oxidative stress; SSRI, selective serotonin-reuptake inhibitor, VD, vascular disruption.
[a] Not all specific birth defects were assessed in all live-born infants

were valproic acid (36,709 cases from 2 studies), carbamazepine (11,872 cases from 1 study), diazepam (3,703 cases from 3 studies), promethazine (3,094 cases from 1 study), and oral contraceptives (3,038 cases from 1 study)

Appendixes 3 2-3 5 provide the details of the results from all studies incorporated in the systematic review, including prevalence estimates from the cohort studies and ORs with 95% CIs from the case-control studies Although some differences with regard to statistical significance were observed depending on the reference population used, 24 drugs or drug groups, in particular acetaminophen, antihypertensive drugs, antiepileptic drugs, aspirin, sex hormones, and SSRIs, were associated with increased prevalences of a number of specific birth defects in the population-based cohort studies In the cohorts from voluntary reporting systems, this was the case for 16 specific drugs or drug groups, with only fluoxetine, statins, and valproic acid being associated with five or more different defects Within the case-control studies with population controls or malformed controls, increased odds for specific birth defects were observed for 30 and 13 drugs or drug groups, respectively, but these results were not always consistent when multiple case-control studies were conducted on a particular drug-birth defect association Again, several associations were seen for the drugs mentioned above, but also for benzodiazepines, NSAIDs, and promethazine Below we describe the associations that were observed in at least two study design categories in more detail

In the population-based cohort studies, an increased prevalence of cleft palate was observed in relation to acetaminophen use (16 62 vs 4 90 or 5 45 per 10,000 live births, p<0 001),[19 20] which was also found in a population-based case-control study (adjusted OR 3 7, 95% CI 1 1-12 0),[21] but refuted in two other studies [22 23] Based on 5,560 infants exposed to NSAIDs, an increased prevalence of ventricular septal defects was found (52 16 vs 26 35 or 36 67 per 10,000 live births, p<0 001) [24 25] NSAID use was also associated with ventricular septal defects in an English case-control (crude OR 4 2, 95% CI 1 5-14 3),[26] but no association with muscular ventricular septal defects was observed in an American case-control study [27] Aspirin use was assessed in one population-based cohort study, which showed – among others – increased prevalences of omphalocele (6 73 per 10,000 live births, p≤0 004) and limb reduction defects (7 40 per 10,000), but the latter only when compared with the European reference population (p=0 03) [28] In case-control studies with population controls, aspirin use also seemed to be associated with omphalocele (adjusted OR 1 6, 95% CI 0 9-3 1)[29] and limb reduction defects (adjusted OR 4 2, 95% CI 0 9-19 9) [30]

Table 3.5 Summary of the case-control studies included in the systematic review

Drug	Mechanism	Case-control studies with population controls		Case-control studies with malformed controls	
		No. of studies	No. of cases	No. of studies	No. of cases
Acetaminophen	OS	5	14,153	3	1,822
Antihypertensive medication	VD	4	6,858	0	0
ACE inhibitor	VD+AT	1	758	0	0
AT II blocker	VD+AT	1	758	0	0
Atenolol	VD	1	758	0	0
Calcium-channel blocker	VD	2	6,175	0	0
Furosemide	VD	1	2,958	0	0
Methyldopa	VD	1	758	0	0
Metoprolol	VD	1	601	0	0
Oxprenolol	VD	1	1,975	1	1,374
Propranolol	VD	1	1,769	0	0
Benzodiazepines	OS+GA	1	1,044	1	826
Chlordiazepoxide	OS+GA	3	6,791	0	0
Diazepam	OS+GA	3	6,960	3	3,703
Nitrazepam	OS+GA	1	809	0	0
Oxazepam	OS+GA	0	0	1	277
Carbamazepine	FA	1	601	1	11,872
Dextromethorphan	NA	2	1,494	1	332
Doxycycline	OS	1	3,405	0	0
Ephedrine	VD	1	381	0	0
Epinephrine	VD	1	381	0	0
Lamotrigine	FA	0	0	1	1,943
Metronidazole	OS	2	6,924	1	1,374
Misoprostol	VD	1	452	0	0
Nitrofurantoin	OS	2	5,810	1	1,374
NSAIDs	VD+CI	5	2,391	0	0
Ibuprofen	VD+CI	3	1,095	1	332
Naproxen	VD+CI	2	1,697	0	0
Oxytetracycline	OS	2	5,359	0	0
Phenobarbital	FA+GA	1	1,975	1	1,374
Phenytoin	FA	1	1,374	1	1,374
Promethazine	OS	2	11,130	1	3,094
Salbutamol	VD	2	3,010	1	294
Salicylates	HI	2	487	1	327
Aspirin	OS+HI+CI	8	8,351	4	2,640
Salmeterol	VD	1	381	0	0
Sex hormones	ED	3	1,069	2	932
17OHP	ED	3	5,898	1	1,374
Allylestrenol	ED	1	1,975	1	1,374
Fertility treatment	ED	3	1,943	0	0
Clomiphene	ED	5	21,024	2	973
hCG	ED	1	4,960	2	1,699
Progestin	ED	1	500	0	0
Horm. pregn. test	ED	2	371	0	0
Oral contraceptives	ED	9	16,149	1	3,038
Estrogen	ED	2	381	0	0
SSRIs	5-HT	2	11,171	0	0
Citalopram	5-HT	1	1,923	0	0
Fluoxetine	5-HT	2	6,728	0	0
Paroxetine	5-HT	2	6,728	1	183
Sertraline	5-HT	2	6,728	0	0
Sulfasalazine	FA	2	2,413	0	0
Terbutaline	OS+VD	1	1,975	1	1,374

Table 3.5 (Continued)

Drug	Mechanism	Case-control studies with population controls		Case-control studies with malformed controls	
		No. of studies	No. of cases	No. of studies	No. of cases
Triptans	VD	1	514	0	0
Valproic acid	FA+OS+HI	1	2,375	2	36,709
Vitamin A	NC	0	0	1	542

5-HT, influence serotonin signaling, 17OHP, 17-hydroxyprogesterone, ACE, angiotensin-converting enzyme, AT II, angiotensin II, FA, folate antagonism, GA, GABA receptor antagonist, hCG, human chorionic gonadotropin, HI, HDAC inhibition, horm pregn test, hormonal pregnancy test, NC, neural crest cell disruption, NSAID, non-steroidal anti-inflammatory drug, OS, oxidative stress, SSRI, selective serotonin-reuptake inhibitor, VD, vascular disruption

Antihypertensive drugs were associated with three types of cardiovascular defects in a population-based cohort study and a case-control study with population controls,[31 32] namely ventricular septal defects (153.85 per 10,000, p<0.001; adjusted OR 1.7, 95% CI 0.8-3.5), atrial septal defects (83.92 per 10,000, p<0.001; adjusted OR 2.4, 95% CI 1.3-4.4), and coarctation of the aorta (20.98 per 10,000, p<0.001; adjusted OR 3.0, 95% CI 1.3-6.6). In the same case-control study, an increased risk of pulmonary valve stenosis was also observed (adjusted OR 2.6, 95% CI 1.3-5.4), but the type of antihypertensive medication used was not specified. Based on two population-based cohort studies, an increased prevalence of this defect was seen in particular in relation to use of angiotensin-converting enzyme inhibitors (111.94 per 10,000, p<0.001).[33 34] With respect to non-cardiac defects, antihypertensive medication was associated with an increased prevalence of hypospadias when compared to the European reference population (96.02 per 10,000, p<0.001).[31] An increased crude OR for severe hypospadias was also observed in relation to use of antihypertensive medication in an American case-control study, but the OR diminished after correction for confounding factors (adjusted OR 1.4, 95% CI 0.7-2.9).[35]

As a group, SSRIs were associated with craniosynostosis with a prevalence of 9.15 per 10,000 (p≤0.03) in a population-based cohort study and an adjusted OR of 2.5 (95% CI 1.5-4.0) in a case-control study with population controls.[36 37] When looking at individual SSRIs, this association was only observed for fluoxetine in cohorts studies from voluntary reporting systems (46.66 per 10,000, p<0.001)[38 42] and in the same case-control study (adjusted OR 2.8, 95% CI 1.3-6.1),[37] in which the OR for paroxetine also seemed to be increased (adjusted OR 2.3, 95% CI 0.8-6.4). However, a second case-control study with population controls did not show an association between SSRIs and craniosynostosis (adjusted OR 0.8, 95% CI 0.2-3.5).[43] In the population-based cohort studies, fluoxetine and paroxetine were also

associated with ventricular septal defects (54.45 per 10,000, p≤0.03; and 81.23 per 10,000, p<0.001, respectively) and with atrial septal defects when compared with the U.S. reference population (39.50 per 10,000, p<0.001; and 35.91 per 10,000, p<0.001, respectively).[44 46] The same associations were found in the cohort studies from voluntary reporting systems,[38 42] but the associations between individual SSRIs and cardiovascular defects were not assessed in case-control studies. Fluoxetine was also associated with esophageal atresia based on one population-based cohort study and one case-control study with population controls (13.17 per 10,000, p≤0.004; adjusted OR 2.4, 95% CI 0.9-6.4)[37,46] and with hypospadias in both cohort study designs when compared with the European reference population (64.52 and 91.46 per 10,000, p≤0.04).[38 42,46]

For three anti-epileptic drugs, namely carbamazepine, phenobarbital, and valproic acid, associations with a number of specific birth defects were observed. Based on the population-based cohort studies, an increased prevalence after carbamazepine use was found for hypospadias (128.76 per 10,000, p≤0.002).[47 58] Although no increased risk was observed for hypospadias in case-control studies, an increased prevalence of this defect was found among the 779 carbamazepine-exposed male infants included in cohort studies from voluntary reporting systems (115.53 per 10,000; p≤0.04).[59 61] Phenobarbital was associated with cleft lip ± cleft palate (53.76 per 10,000, p≤0.006) and polydactyly (71.63 per 10,000, p<0.001) in the data from several population-based cohort studies.[28,48 49,51,53,54,62 65] The association with polydactyly was also observed in cohort studies from voluntary reporting systems (68.08 per 10,000, p≤0.02),[61 66] whereas the increased risk of cleft lip ± cleft palate was only found in a case-control study with population controls.[21] In three study designs (the two types of cohort studies and the case-control studies with malformed controls), valproic acid was associated with a number of defects: spina bifida, atrial septal defects, cleft palate, hypospadias, polydactyly, and craniosynostosis.[41,48,49,53,55-58,67 73] In case-control studies with population controls, only the association between this anti-epileptic drug and hypospadias was assessed, showing a borderline increased risk (crude OR 2.9, 95% CI 0.9-8.4).[74]

In an American case-control study with population controls,[75] clomiphene use was found to increase the risk of multiple defects. For two of these defects, increased risks were also observed in a case-control study with malformed controls, namely for anencephaly (population controls: adjusted OR 2.3, 95% CI 1.1-4.7; malformed controls: crude OR 3.7, 95% CI 1.5-9.2) and atrial septal defects (population controls: adjusted OR 1.5, 95% CI 1.0-2.3; malformed controls: crude OR 3.1, 95% CI 1.6-5.9). However, an English case-control study with population controls could not confirm the increased risk of anencephaly (adjusted OR 0.8, 95% CI 0.3-2.7).[76]

Discussion

This study aimed to obtain more insight in the use of medical drugs that are considered to be associated with teratogenic mechanisms by determining their prescription rates among pregnant Dutch women and conducting a systematic review of epidemiologic studies of their effects. The results showed that in a substantial proportion of pregnancies in our study population at least one drug associated with a teratogenic mechanism was dispensed in the first trimester. Furthermore, the prescription rates increased over time for vasoactive drugs, SSRIs, and serotonin receptor agonists/antagonists. Epidemiologic studies that assessed their teratogenic risks were identified for less than half of the drugs included. For a number of drugs, including acetaminophen, antihypertensive medication, aspirin, carbamazepine, clomiphene, NSAIDs, phenobarbital, SSRIs, in particular fluoxetine and paroxetine, and valproic acid, associations between exposure in early pregnancy and specific birth defects were observed, but for most drugs and drug groups, the numbers of exposed infants were too small to draw any conclusions regarding their human teratogenic risks.

Study strengths and limitations
The strength of our drug utilization study is that the population-based prescription database used, the IADB.nl database, covers a relatively large and well-defined population in the Netherlands. The data are recorded prospectively and cover prescriptions from different health care professionals. However, actual use of the medication prescribed is unknown, so non-compliance could have led to overestimation of exposure prevalences. For some drugs, exposure has been underestimated as the IADB.nl database does not include drugs that are available over-the-counter, drugs administered during hospitalization, and medication that is delivered directly to the patient by the manufacturer (e.g., tumor necrosis factor alpha). As only part of the pregnant women is identified in the 'Pregnancy IADB', selection bias may also occur. Due to the methodology used to identify pregnant women, women with a miscarriage, induced abortion, or stillbirth or women whose child did not survive until the first prescription are not included. As assumptions were made on the length of the gestational period, the estimated gestational age may lead to misclassification for the trimesters in which the drug was prescribed.

For at least two of the teratogenic mechanisms, endocrine disruption and inhibition of histone deacetylase, prescription drug exposure was underestimated due to the fact that not all drugs could be included. A number of drugs have a coating that contains phthalates,[77] which may affect male reproductive development due to endocrine disrupting effects.[78] However, the specific drugs marketed in the Netherlands that have such a coating are unknown, leading to underestimation of prescriptions for

drugs with endocrine disrupting potential. In addition, it is unknown which drugs contain boric acid as an inactive ingredient, resulting in underestimation of the number of women with prescriptions for potential histone deacetylase inhibitors.

Despite our exhaustive search techniques in the systematic review, some potentially relevant studies might have been missed due to the exclusion of non-English language articles and publication bias. In addition, our focus on the risks of specific birth defects instead of the overall occurrence of congenital malformations led to the exclusion of 99 papers. These studies may or may not have found increased risks of birth defects overall, but increases in the prevalence of specific birth defects were not addressed and could easily have been missed in these studies. The same argument applies to restricting the exposure to specific medical drugs. As a result, a small number of papers that combined certain drugs into one exposure variable based on their proposed teratogenic mechanism (e.g., folate antagonism)[79 81] were not included. Furthermore, the decision to exclude case reports and case series resulted in excluding the landmark papers that identified the teratogenic properties of thalidomide and isotretinoin.[82 84]

As with all systematic reviews and meta-analyses, the validity of our results is limited by the validity and reporting of the studies from which the original data were extracted. To increase the validity of our study, we divided the studies that were included into four study design categories. As cohort studies originating from voluntary reporting systems are prone to selection bias,[85,86] the results from these studies were separated from those of population-based cohort studies. Comparatively, case-controls studies with population controls (prone to recall bias) were separated from those with malformed control subjects (prone to selection bias). For the cohort studies, this approach decreased the study power to detect increased prevalences of specific birth defects in association with the drugs and drug groups selected. However, as the case-control studies were not pooled, our approach did not affect the ability to detect associations in epidemiologic studies with this design.

Implications
When considering the results from our drug utilization study and systematic review together, it is cause for concern that the drugs most often dispensed in the first trimester of pregnancy are not necessarily the drugs for which teratogenic risks were assessed in the literature. Studies on oxidative stress inducers were sparse and often had small sample sizes, while this was the drug group for which the highest first trimester prescription rate was observed. More specifically, no epidemiologic studies on the human teratogenic risks of iron preparations, with a first trimester prescription rate of 39.6 per 1,000 pregnancies, were identified. This was also the case, although

to a somewhat lesser extent, for vasoactive drugs and COX inhibitors. In contrast, many of the included studies determined the teratogenic potential of antiepileptic drugs, for which the prescription rate in the first trimester was only 2.3 per 1,000 pregnancies. However, the trend of increasing prescription rates for SSRIs, which was also observed in an American study,[87] seems to be accompanied by increasing numbers of studies on their teratogenic risks.

Overall, the numbers of subjects included in epidemiologic studies on the teratogenicity of medical drugs were small, especially in light of the prevalence of exposure. For only 21 drugs or drug groups, more than 1,000 exposed live-born infants were included in cohort studies. Although based on small sample sizes, a number of drugs was associated with strong increases in the prevalence of specific birth defects or yielded statistically significant OR estimates. For many drugs, an increased risk was observed in only one study design and was not confirmed in other studies for two reasons: (1) the association was not assessed in other study designs, or (2) due to small samples sizes, the differences were not statistically significant.

In conclusion, our study confirms the lack of knowledge on the teratogenic effects of medical drugs that was reported previously,[2] although many of these drugs are commonly dispensed in the first trimester. Current knowledge on the teratogenic risks of medical drugs is not at all associated with their prescription rates: many uncertainties exist on the fetal safety of drugs that are frequently dispensed, while the adverse effects of drugs that are less often used, but are already known to increase the risks of specific birth defects, are still being studied. These studies may yield important information from a mechanism-based point of view, but from a public health perspective, it is more important to study prevalent exposures to potentially benefit the largest number of people from future generations by primary prevention. Therefore, large-scale epidemiologic studies are needed in order to enable prescribers to make evidence-based decisions regarding pharmacologic treatment options in pregnancy.

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Appendix 3.1 Articles included in the systematic review.

Population-based cohort studies

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Appendix 3.2 Results of the population-based cohort studies.

| Drug | Birth defect | No. of infants | | Prevalence | P-value | | Reference |
		Exposed	Affected		Europe	U.S.	
5-Fluorouracil	N/A	4	0	–	–	–	Van Le *et al.*, 1991
Acetaminophen	Spina bifida	26,479	7	2.64	0.45	0.04	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Cataract	26,479	8	3.02	0.002	0.34	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Ventricular septal defect	26,479	95	35.88	0.003	0.001	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Cleft palate	26,479	44	16.62	<0.001	<0.001	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Small intestinal atresia	26,479	6	2.27	<0.001	0.43	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Hirschsprung's disease	26,479	15	5.66	<0.001	<0.001	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Gastroschisis	26,479	3	1.13	0.40	0.33	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Hypospadias	13,504	80	59.24	<0.001	0.99	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Acetaminophen	Craniosynostosis	26,479	33	12.46	<0.001	<0.001	Thulstrup *et al.*, 1999; Rebordosa *et al.*, 2008
Amiodarone	Ventricular septal defect	6	1	1666.67	–	–	Plomp *et al.*, 1991; Ovadia *et al.*, 1994
Antihypertensive	Spina bifida	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Cataract	1,430	2	13.99	<0.001	0.002	Lennestål *et al.*, 2009
Antihypertensive	Glaucoma	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Anotia	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Common truncus arteriosus	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Ventricular septal defect	1,430	22	153.85	<0.001	<0.001	Lennestål *et al.*, 2009
Antihypertensive	Atrial septal defect	1,430	12	83.92	<0.001	<0.001	Lennestål *et al.*, 2009
Antihypertensive	Aortic valve atresia/stenosis	1,430	3	20.98	<0.001	<0.001	Lennestål *et al.*, 2009
Antihypertensive	Hypoplastic left heart	1,430	3	20.98	<0.001	<0.001	Lennestål *et al.*, 2009
Antihypertensive	Coarctation of aorta	1,430	3	20.98	<0.001	<0.001	Lennestål *et al.*, 2009
Antihypertensive	Cleft lip ± cleft palate	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Cleft palate	1,430	2	13.99	0.12	0.17	Lennestål *et al.*, 2009
Antihypertensive	Small intestinal atresia	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Anorectal malformation	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Hypospadias	729	7	96.02	<0.001	0.20	Lennestål *et al.*, 2009
Antihypertensive	Polydactyly	1,430	2	13.99	0.34	0.88	Lennestål *et al.*, 2009
Antihypertensive	Syndactyly	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
Antihypertensive	Craniosynostosis	1,430	1	6.99	–	–	Lennestål *et al.*, 2009
ACE inhibitors	Spina bifida	268	1	37.31	–	–	Cooper *et al.*, 2006; Karthikeyan *et al.*, 2011
ACE inhibitors	Ventricular septal defect	268	3	111.94	0.006	0.006	Cooper *et al.*, 2006; Karthikeyan *et al.*, 2011

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
ACE inhibitors	Atrial septal defect	268	6	223.88	<0 001	<0.001	Cooper *et al.*, 2006, Karthikeyan *et al.*, 2011
ACE inhibitors	Pulmonary valve stenosis	268	3	111.94	<0.001	<0 001	Cooper *et al.*, 2006, Karthikeyan *et al.*, 2011
ACE inhibitors	Choanal atresia	268	1	37.31	–	–	Cooper *et al.*, 2006; Karthikeyan *et al.*, 2011
ACE inhibitors	Hirschsprung's disease	268	1	37.31	–	–	Cooper *et al.*, 2006, Karthikeyan *et al.*, 2011
ACE inhibitors	Diaphragmatic hernia	268	1	37 31	–	–	Cooper *et al.*, 2006, Karthikeyan *et al*, 2011
ACE inhibitors	Hypospadias	137	2	145 99	0.006	0.19	Cooper *et al.*, 2006, Karthikeyan *et al.*, 2011
Captopril	N/A	9	0	–	–	–	Burrows and Burrows, 1998
Enalapril	N/A	9	0	–	–	–	Burrows and Burrows, 1998
Lisinopril	N/A	9	0	–	–	–	Burrows and Burrows, 1998
AT II blockers	Polydactyly	30	1	333.33	–	–	Serreau *et al.*, 2005, Gersak *et al.*, 2009; Karthikeyan *et al.*, 2011
AT II blockers	Craniosynostosis	30	1	333.33	–	–	Serreau *et al.*, 2005, Gersak *et al.*, 2009, Karthikeyan *et al.*, 2011
Acebutolol	N/A	6	0	–	–	–	Dubois *et al.*, 1982
Atenolol	N/A	5	0	–	–	–	Dubois *et al.*, 1982
Ca-channel block	N/A	25	0	–	–	–	Sørensen *et al.*, 1998
Felodipine	N/A	3	0	–	–	–	Casele *et al.*, 1997
Pindolol	N/A	7	0	–	–	–	Dubois *et al.*, 1982
Sotalol	N/A	1	0	–	–	–	O'Hare *et al.*, 1980
Benzodiazepines	Ventricular septal defect	1,391	6	43 13	0.22	0.17	Bergman *et al.*, 1992, Oberlander *et al.*, 2008
Benzodiazepines	Atrial septal defect	1,391	2	14.38	0 67	0 44	Bergman *et al.*, 1992; Oberlander *et al.*, 2008
Benzodiazepines	Limb reduction	64	1	156 25	–	–	Bergman *et al.*, 1992, Oberlander *et al.*, 2008
Benzodiazepines	Syndactyly	64	1	156.25	–	–	Bergman *et al.*, 1992; Oberlander *et al.*, 2008
Alprazolam	N/A	6	0	–	–	–	Gidai *et al.*, 2008c
Chlordiazepoxide	Atrial septal defect	18	1	555 56	–	–	Gidai *et al.*, 2008b
Clonazepam	Tetralogy of Fallot	46	1	217 39	–	–	Robert *et al.*, 1986; Canger *et al.*, 1999; Holmes *et al.*, 2001; Lin *et al.*, 2004
Diazepam	N/A	39	0	–	–	–	Robert *et al.*, 1986; Battino *et al.*, 1992; Gidai *et al.*, 2008a
Lorazepam	N/A	1	0	–	–	–	Holmes *et al.*, 2001

| Drug | Birth defect | No. of infants | | Prevalence | P-value | | Reference |
		Exposed	Affected		Europe	U.S.	
Cabergoline	Atrial septal defect	173	1	57 80	–	–	Robert *et al*, 1996, Verhelst *et al*, 1999
Cabergoline	Craniosynostosis	173	1	57 80	–	–	Robert *et al*, 1996, Verhelst *et al*, 1999
Carbamazepine	Spina bifida	667	1	14 99	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juárez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Ventricular septal defect	667	1	14 99	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Tetralogy of Fallot	667	1	14 99	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Cleft lip ± cleft palate	667	1	14 99	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juárez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Cleft palate	667	1	14 99	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez Olguin *et al*, 2008, Mawer *et al*, 2010

Appendix 3.2 (Continued)

Drug	Birth defect	No of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U S.	
Carbamazepine	Esophageal atresia	667	2	29 99	<0 001	<0 001	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Diaphragmatic hernia	1,370	1	7 30	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Wide *et al*, 2004, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Hypospadias	699	9	128 76	<0 001	0 02	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Wide *et al*, 2004, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Limb reduction	667	1	14 99	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Carbamazepine	Craniosynostosis	1,370	1	7 30	–	–	Kuhnz *et al*, 1983, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Omtzigt *et al*, 1993, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Wide *et al*, 2004, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Chlorpromazine	Syndactyly	57	1	175 44	–	–	Rumeau Rouquette *et al* 1977
Clomipramine	Transposition of great vessels	1,029	2	19 44	0 001	<0 001	Kallen and Otterblad Olausson, 2006

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Cyclosporine	N/A	63	0	–	–	–	Ghanem *et al*, 2005; Christopher *et al*, 2006
Ergotamine	Ventricular septal defect	213	3	140 85	0.001	0 001	Kallén and Lygner, 2001
Isoniazid	N/A	17	0	–	–	–	Marcus, 1967
Lamotrigine	Ventricular septal defect	135	1	74.07	–	–	Meador *et al*, 2006, Mawer *et al*, 2010
Mercaptopurine	N/A	11	0	–	–	–	Francella *et al*, 2003, Nørgård *et al*, 2003
Metformin	Cleft palate	327	1	30.58	–	–	Glueck *et al*, 2004, Thatcher and Jackson, 2006; Turner *et al*, 2006
Methotrexate	N/A	6	0	–	–	–	Kozlowski *et al*, 1990, Fragoso *et al*, 2009
Metoclopramide	Spina bifida	189	1	52.91	–	–	Sørensen *et al*, 1999
Metoclopramide	Atrial septal defect	189	1	52 91	–	–	Sørensen *et al.*, 1999
Metoclopramide	Pulmonary valve stenosis	189	1	52.91	–	–	Sørensen *et al*, 1999
Metoclopramide	Hypospadias	96	1	104 17	–	–	Sørensen *et al*, 1999
Metronidazole	Transposition of great vessels	102	1	98 04	–	–	Perl, 1965; Robinson and Mirchandani, 1965; Sørensen *et al*, 1999
Metronidazole	Ventricular septal defect	102	1	98.04	–	–	Perl, 1965; Robinson and Mirchandani, 1965; Sørensen *et al*, 1999
Mianserin	Anorectal malformation	63	1	158 73	–	–	Lennestål and Kallén, 2007
Mirtazapine	Ventricular septal defect	154	2	129 87	0 01	0 01	Lennestål and Kállén, 2007
Mirtazapine	Atrial septal defect	154	1	64.94	–	–	Lennestål and Kallén, 2007
Mirtazapine	Cleft palate	154	1	64 94	–	–	Lennestål and Kallén, 2007
Mirtazapine	Hypospadias	79	1	126 58	–	–	Lennestål and Kállén, 2007
Misoprostol	Spina bifida	118	1	84 75	–	–	Dal Pizzol *et al*, 2008
Misoprostol	Syndactyly	118	1	84.75	–	–	Dal Pizzol *et al*, 2008
Nitrofurantoin	N/A	32	0	–	–	–	Hailey *et al*, 1983
NSAIDs	Transposition of great vessels	5,560	3	5 40	0.25	0.04	Ericson and Kallén, 2001, van Gelder *et al*, 2011
NSAIDs	Ventricular septal defect	5,560	29	52 16	<0 001	<0.001	Ericson and Kállén, 2001, van Gelder *et al.*, 2011
NSAIDs	Atrial septal defect	5,560	17	30 58	0 06	<0 001	Ericson and Kallén, 2001; van Gelder *et al*, 2011
NSAIDs	Atrioventricular septal defect	2,557	1	3.91	–	–	Ericson and Kallén, 2001

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
NSAIDs	Tetralogy of Fallot	5,560	1	1 80	–	–	Ericson and Kallen, 2001, van Gelder *et al*, 2011
NSAIDs	Ebstein's anomaly	2,557	1	3 91	–	–	Ericson and Kallen, 2001
NSAIDs	Pulmonary valve stenosis	2,557	1	3 91	–	–	Ericson and Kallen, 2001
NSAIDs	Aortic valve atresia/stenosis	2,557	1	3 91	–	–	Ericson and Kallen, 2001
NSAIDs	Coarctation of aorta	5,560	2	3 60	0 78	0 70	Ericson and Kallen, 2001, van Gelder *et al*, 2011
NSAIDs	Choanal atresia	2,557	1	3 91	–	–	Ericson and Kallen, 2001
NSAIDs	Cleft lip ± cleft palate	5,560	6	10 79	0 40	0 77	Ericson and Kallen, 2001, van Gelder *et al*, 2011
NSAIDs	Cleft palate	5,560	1	1 80	–	–	Ericson and Kallen, 2001, van Gelder *et al*, 2011
NSAIDs	Anorectal malformation	5,560	2	3 60	0 49	0 97	Ericson and Kallen, 2001, van Gelder *et al*, 2011
NSAIDs	Gastroschisis	5,560	1	1 80	–	–	Ericson and Kallen, 2001, van Gelder *et al*, 2011
NSAIDs	Hypospadias	1,304	5	38 34	0 38	0 32	Ericson and Kallen, 2001
NSAIDs	Limb reduction	2,257	3	11 73	0 02	0 11	Ericson and Kallen, 2001
NSAIDs	Polydactyly	2,257	2	7 82	0 77	0 42	Ericson and Kallen, 2001
NSAIDs	Syndactyly	2,257	1	3 91	–	–	Ericson and Kallen, 2001
Oxomemazine	Cleft lip ± cleft palate	14	1	714 29	–	–	Rumeau-Rouquette *et al*, 1977
Phenobarbital	Spina bifida	400	1	25 00	–	–	Fedrick, 1973, Lowe, 1973, Annegers *et al*, 1974, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Waters *et al*, 1994, Canger *et al*, 1999, Holmes *et al*, 2001, Timmermann *et al*, 2009
Phenobarbital	Ventricular septal defect	1,787	10	55 96	0 01	0 18	Fedrick, 1973, Lowe, 1973, Heinonen *et al*, 1977, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Waters *et al*, 1994, Canger *et al*, 1999, Holmes *et al*, 2001, Timmermann *et al*, 2009

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Phenobarbital	Tetralogy of Fallot	372	2	53.76	<0.001	<0.001	Fedrick, 1973, Lowe, 1973, Bertollini *et al* , 1987; Kaneko *et al* , 1988; Waters *et al* , 1994, Canger *et al* , 1999, Holmes *et al.*, 2001, Timmermann *et al.*, 2009
Phenobarbital	Coarctation of aorta	1,787	4	22.38	<0.001	<0 001	Fedrick, 1973; Lowe, 1973; Heinonen *et al* , 1977, Bertollini *et al* , 1987, Kaneko *et al.*, 1988, Waters *et al* , 1994; Canger *et al* , 1999; Holmes *et al.*, 2001; Timmermann *et al.*, 2009
Phenobarbital	Cleft lip ± cleft palate	372	2	53.76	0 001	0 006	Fedrick, 1973; Lowe, 1973, Bertollini *et al* , 1987, Kaneko *et al.*, 1988; Waters *et al* , 1994, Canger *et al* , 1999; Holmes *et al* , 2001; Timmermann *et al* , 2009
Phenobarbital	Cleft palate	372	1	26.88	–	–	Fedrick, 1973; Lowe, 1973; Bertollini *et al.*, 1987; Kaneko *et al* , 1988; Waters *et al* , 1994; Canger *et al* , 1999, Holmes *et al* , 2001; Timmermann *et al* , 2009
Phenobarbital	Diaphragmatic hernia	372	1	26.88	–	–	Fedrick, 1973; Lowe, 1973, Bertollini *et al* , 1987, Kaneko *et al* , 1988, Waters *et al* , 1994, Canger *et al* , 1999, Holmes *et al* , 2001; Timmermann *et al* , 2009
Phenobarbital	Hypospadias	190	1	52 63	–	–	Fedrick, 1973, Lowe, 1973, Bertollini *et al* , 1987, Kaneko *et al* , 1988, Waters *et al.*, 1994, Canger *et al* , 1999, Holmes *et al* , 2001, Timmermann *et al* , 2009
Phenobarbital	Limb reduction	372	1	26.88	–	–	Fedrick, 1973; Lowe, 1973, Bertollini *et al* , 1987, Kaneko *et al.*, 1988, Waters *et al.*, 1994; Canger *et al.*, 1999; Holmes *et al* , 2001, Timmermann *et al* , 2009

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Phenobarbital	Polydactyly	1,815	13	71 63	<0 001	<0 001	Fedrick, 1973, Lowe, 1973, Annegers *et al*, 1974, Heinonen *et al*, 1977, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Waters *et al*, 1994, Canger *et al*, 1999, Holmes *et al*, 2001, Timmermann *et al*, 2009
Phenytoin	Microphthalmia	527	1	18 98	–	–	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Annegers *et al*, 1974, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Kini *et al*, 2006, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008
Phenytoin	Ventricular septal defect	503	3	59 64	0 15	0 39	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Kini *et al*, 2006, Meador *et al*, 2006, Juárez-Olguin *et al*, 2008
Phenytoin	Hypoplastic left heart	503	1	19 88	–	–	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Kini *et al*, 2006, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008
Phenytoin	Cleft palate	527	1	18 98	–	–	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Annegers *et al*, 1974, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Waters *et al*, 1994, Nulman *et al*, 1997, Canger *et al*, 1999, Holmes *et al*, 2001, Kini *et al*, 2006, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Phenytoin	Duodenal atresia	527	1	18 98	–	–	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Annegers et al, 1974, Bertollini et al, 1987, Kaneko et al, 1988, Waters et al, 1994, Nulman et al, 1997, Canger et al, 1999, Holmes et al, 2001, Kini et al, 2006, Meador et al, 2006, Juarez-Olguin et al, 2008
Phenytoin	Gastroschisis	527	1	18 98	–	–	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Annegers et al, 1974, Bertollini et al, 1987, Kaneko et al, 1988, Waters et al, 1994, Nulman et al, 1997, Canger et al, 1999, Holmes et al, 2001, Kini et al, 2006, Meador et al, 2006, Juarez-Olguin et al, 2008
Phenytoin	Hypospadias	269	4	148 70	<0 001	0 06	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Annegers et al, 1974, Bertollini et al, 1987, Kaneko et al, 1988, Waters et al, 1994, Nulman et al, 1997, Canger et al, 1999, Holmes et al, 2001, Kini et al, 2006, Meador et al, 2006, Juarez-Olguin et al, 2008
Phenytoin	Limb reduction	527	1	18 98	–	–	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Annegers et al, 1974, Bertollini et al, 1987, Kaneko et al, 1988, Waters et al, 1994, Nulman et al, 1997, Canger et al, 1999, Holmes et al, 2001, Kini et al, 2006, Meador et al, 2006, Juarez-Olguin et al, 2008
Phenytoin	Polydactyly	527	1	18 98	–	–	Watson and Spellacy, 1971, Fedrick, 1973, Lowe, 1973, Annegers et al, 1974, Bertollini et al, 1987, Kaneko et al, 1988, Waters et al, 1994, Nulman et al, 1997, Canger et al, 1999, Holmes et al, 2001, Kini et al, 2006, Meador et al, 2006, Juarez-Olguin et al, 2008

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Pizotifen	N/A	12	0	–	–	–	Kallén and Lygner, 2001
Primidone	Ventricular septal defect	43	1	232 56	–	–	Lowe, 1973, Kaneko *et al*, 1988; Canger *et al*, 1999
Primidone	Cleft lip ± cleft palate	86	1	116 28	–	–	Lowe, 1973; Kaneko *et al*, 1988, Canger *et al*, 1999
Primidone	Hypospadias	44	1	227.27	–	–	Lowe, 1973, Kaneko *et al.*, 1988, Canger *et al.*, 1999
Promethazine	Transposition of great vessels	11	1	909 09	–	–	Petik *et al.*, 2008
Promethazine	Ventricular septal defect	11	1	909.09	–	–	Petik *et al* 2008
Promethazine	Diaphragmatic hernia	2,775	2	7.21	0.05	0.09	Kallén, 2002; Petik *et al* 2008
Promethazine	Hypospadias	1,415	1	7 07	–	–	Kallén, 2002, Petik *et al*, 2008
Salbutamol	Hypoplastic left heart	648	1	15.43	–	–	Correy *et al*, 1991
Salicylates	Ventricular septal defect	146	3	205 48	<0 001	<0 001	Turner and Collins, 1975
Salicylates	Atrial septal defect	146	1	68.49	–	–	Turner and Collins, 1975
Salicylates	Hypoplastic left heart	146	1	68.49	–	–	Turner and Collins, 1975
Salicylates	Diaphragmatic hernia	146	1	68.49	–	–	Turner and Collins, 1975
Aspirin	Anencephaly	14,864	10	6.73	<0.001	0.05	Heinonen *et al*, 1977
Aspirin	Spina bifida	14,864	19	12 78	<0 001	<0 001	Heinonen *et al*, 1977
Aspirin	Anophthalmia/microphthalmia	14,864	5	3 36	0 16	–	Heinonen *et al*, 1977
Aspirin	Cataract	14,864	17	11.44	0.02	<0 001	Heinonen *et al*, 1977
Aspirin	Transposition of great vessels	14,864	7	4.71	0 003	0 006	Heinonen *et al*, 1977
Aspirin	Ventricular septal defect	14,864	54	36.33	0.02	0.01	Heinonen *et al.*, 1977
Aspirin	Atrial septal defect	14,864	13	8.75	0.003	0 16	Heinonen *et al*, 1977
Aspirin	Atrioventricular septal defect	14,864	10	6.73	<0.001	0 003	Heinonen *et al*, 1977
Aspirin	Tetralogy of Fallot	14,864	8	5 38	0.02	0.15	Heinonen *et al*, 1977
Aspirin	Aortic valve atresia/stenosis	14,864	8	5 38	<0.001	<0.001	Heinonen *et al*, 1977
Aspirin	Coarctation of aorta	14,864	17	11 44	<0.001	<0.001	Heinonen *et al*, 1977
Aspirin	Cleft lip ± cleft palate	14,864	12	8 07	0 85	0.56	Heinonen *et al*, 1977
Aspirin	Cleft palate	14,864	4	2 69	0 22	0.15	Heinonen *et al*, 1977
Aspirin	Anorectal malformation	14,864	13	8 75	<0 001	0.002	Heinonen *et al*, 1977
Aspirin	Omphalocele	14,864	10	6 73	<0.001	0.004	Heinonen *et al*, 1977

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Aspirin	Hypospadias	8,808	64	72 66	<0.001	0.11	Heinonen *et al*, 1977, Correy *et al*, 1991
Aspirin	Limb reduction	14,864	11	7 40	0 03	0 30	Heinonen *et al.*, 1977
Aspirin	Polydactyly	14,864	116	78 04	<0.001	<0 001	Heinonen *et al*, 1977
Aspirin	Syndactyly	14,864	37	24 89	<0.001	–	Heinonen *et al*, 1977
Aspirin	Craniosynostosis	14,864	9	6 05	<0 001	0.19	Heinonen *et al*, 1977
Aspirin	Situs inversus	14,864	7	4.71	<0 001	–	Heinonen *et al.*, 1977
Sex hormones	Transposition of great vessels	1,235	2	16 19	0 005	<0.001	Heinonen *et al*, 1977, Dal Pizzol *et al.*, 2008
Sex hormones	Ventricular septal defect	1,235	8	64.78	0.008	0.007	Heinonen *et al*, 1977, Dal Pizzol *et al.*, 2008
Sex hormones	Atrial septal defect	1,235	2	16 19	0 79	0 14	Heinonen *et al*, 1977, Dal Pizzol *et al.*, 2008
Sex hormones	Tetralogy of Fallot	1,235	1	8.10	–	–	Heinonen *et al.*, 1977, Dal Pizzol *et al*, 2008
Sex hormones	Tricuspid atresia/stenosis	1,235	3	24.29	<0.001	–	Heinonen *et al*, 1977; Dal Pizzol *et al*, 2008
Sex hormones	Ebstein's anomaly	1,235	1	8.10	–	–	Heinonen *et al*, 1977; Dal Pizzol *et al*, 2008
Sex hormones	Aortic valve atresia/stenosis	1,235	4	32.39	<0 001	<0 001	Heinonen *et al.*, 1977; Dal Pizzol *et al.*, 2008
Sex hormones	Choanal atresia	193	1	51.81	–	–	Dal Pizzol *et al*, 2008
Sex hormones	Polydactyly	193	3	155.44	<0.001	<0 001	Dal Pizzol *et al.*, 2008
Sex hormones	Syndactyly	193	1	51 81	–	–	Dal Pizzol *et al.*, 2008
17OHP	N/A	140	0	–	–	–	Varma and Morsman, 1982
Allylestrenol	N/A	27	0	–	–	–	Harlap *et al.*, 1975
Clomiphene	Anencephaly	1,966	1	5 09	–	–	Hack *et al*, 1972, Ahlgren *et al.*, 1976; Harlap, 1976; Gorlitsky *et al.*, 1978; Correy *et al*, 1982; Kurachi *et al.*, 1983; Tulandi *et al*, 2006
Clomiphene	Encephalocele	1,966	1	5 09	–	–	Hack *et al*, 1972; Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978; Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Spina bifida	1,966	1	5 09	–	–	Hack *et al*, 1972, Ahlgren *et al.*, 1976; Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Transposition of great vessels	1,966	1	5 09	–	–	Hack *et al*, 1972, Ahlgren *et al.*, 1976; Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al.*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Clomiphene	Ventricular septal defect	1,966	4	20 34	0 60	0 05	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Atrial septal defect	1,966	2	10 17	0 35	0 78	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Pulmonary valve atresia	1,966	2	10 17	<0 001	<0 001	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Cleft lip ± cleft palate	1,966	1	5 09	–	–	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Cleft palate	1,966	4	20 34	0 002	0 005	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Esophageal atresia	1,966	1	5 09	–	–	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Anorectal malformation	1,966	2	10 17	0 02	0 14	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Omphalocele	1,966	1	5 09	–	–	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Hypospadias	1,003	2	19 94	0 70	0 10	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006
Clomiphene	Limb reduction	1,966	1	5 09	–	–	Hack *et al*, 1972, Ahlgren *et al*, 1976, Harlap, 1976, Gorlitsky *et al*, 1978, Correy *et al*, 1982, Kurachi *et al*, 1983, Tulandi *et al*, 2006

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Clomiphene	Polydactyly	1,966	8	40 69	<0 001	0 005	Hack *et al.*, 1972, Ahlgren *et al.*, 1976, Harlap, 1976; Gorlitsky *et al.*, 1978; Correy *et al.*, 1982; Kurachi *et al.*, 1983; Tulandi *et al.*, 2006
Diethylstilbestrol	Hypospadias	1,053	3	28.49	0.88	0 19	Henderson *et al.*, 1976, Leary *et al.*, 1984
hCG	Ventricular septal defect	345	3	86 96	0.03	0 03	Caspi *et al.*, 1976; Kurachi *et al.*, 1983
hCG	Aortic valve atresia/stenosis	345	1	28 99	–	–	Caspi *et al.*, 1976; Kurachi *et al.*, 1983
hCG	Hypospadias	176	1	56.82	–	–	Caspi *et al.*, 1976; Kurachi *et al.*, 1983
hCG	Syndactyly	345	4	115 94	<0 001	–	Caspi *et al.*, 1976; Kurachi *et al.*, 1983
Horm pregn test	Cataract	661	1	15 13	–	–	Michaelis *et al.*, 1983
Horm. pregn test	Atrial septal defect	661	1	15 13	–	–	Michaelis *et al.*, 1983
Horm. pregn. test	Pulmonary valve atresia	661	1	15.13	–	–	Michaelis *et al.*, 1983
Horm. pregn test	Coarctation of aorta	661	1	15 13	–	–	Michaelis *et al.*, 1983
Horm. pregn test	Cleft lip ± cleft palate	661	3	45.39	<0.001	0.003	Michaelis *et al.*, 1983
OCs	Anencephaly	108	1	92.59	–	–	Harlap and Eldor, 1980
OCs	Ventricular septal defect	958	2	20.88	0.74	0.76	Harlap and Eldor, 1980; Harlap *et al.*, 1985
OCs	Coarctation of aorta	958	2	20.88	0 001	<0.001	Harlap and Eldor, 1980, Harlap *et al.*, 1985
OCs	Cleft lip ± cleft palate	958	2	20.88	0.14	0.26	Harlap and Eldor, 1980; Harlap *et al.*, 1985
OCs	Omphalocele	958	1	10.44	–	–	Harlap and Eldor, 1980; Harlap *et al.*, 1985
OCs	Hypospadias	486	4	82.30	0 02	0.51	Harlap and Eldor, 1980; Harlap *et al.*, 1985
OCs	Polydactyly	958	2	20.88	0 12	0.67	Harlap and Eldor, 1980; Harlap *et al.*, 1985
OCs	Syndactyly	958	1	10.44	–	–	Harlap and Eldor, 1980; Harlap *et al.*, 1985
DMPA	N/A	15	0	–	–	–	Dahlberg, 1982
Levonorgestrel	Cleft lip ± cleft palate	272	1	37.76	–	–	Zhang *et al.*, 2009
Progesterone	Spina bifida	2,324	4	17.21	<0.001	0.02	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Cataract	2,324	1	4.30	–	–	Dillon, 1970; Harlap *et al*, 1975, Katz *et al.*, 1985, Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Common truncus arteriosus	716	1	13.97	–	–	Dillon, 1970, Harlap *et al.*, 1975; Rock *et al.*, 1985, Yovich *et al.*, 1988
Progesterone	Tetralogy of Fallot	716	1	13.97	–	–	Dillon, 1970; Harlap *et al.*, 1975, Rock *et al.*, 1985; Yovich *et al.*, 1988

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Progesterone	Cleft lip ± cleft palate	2,324	3	12.91	0.36	0.60	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Cleft palate	2,324	2	8.61	0.42	0.52	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Duodenal atresia	2,324	1	4.30	–	–	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Diaphragmatic hernia	2,324	1	4.30	–	–	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Bladder exstrophy/epispadias	2,324	2	8.61	<0.001	<0.001	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Hypospadias	2,683	35	126.72	<0.001	<0.001	Dillon, 1970; Harlap *et al.*, 1975; Mau, 1981; Katz *et al.*, 1985; Resseguie *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988; Colvin *et al.*, 2010
Progesterone	Limb reduction	3,312	1	4.30	–	–	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Resseguie *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Polydactyly	2,324	2	8.61	0.81	0.40	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Progesterone	Syndactyly	2,324	1	4.30	–	–	Dillon, 1970; Harlap *et al.*, 1975; Katz *et al.*, 1985; Rock *et al.*, 1985; Yovich *et al.*, 1988
Stilbestrol	Atrial septal defect	2	1	5,000.00	–	–	Harlap *et al.*, 1975
SSRIs	Ventricular septal defect	7,747	24	30.98	0.43	0.41	Oberlander *et al.*, 2008; Kornum *et al.*, 2010; Colvin *et al.*, 2011
SSRIs	Atrial septal defect	7,747	24	30.98	0.02	<0.001	Oberlander *et al.*, 2008; Kornum *et al.*, 2010; Colvin *et al.*, 2011
SSRIs	Atrioventricular septal defect	6,555	2	3.05	0.14	0.84	Källén and Otterblad Olausson, 2007
SSRIs	Tetralogy of Fallot	6,555	1	1.53	–	–	Källén and Otterblad Olausson, 2007
SSRIs	Pulmonary valve stenosis	6,555	1	1.53	–	–	Källén and Otterblad Olausson, 2007
SSRIs	Coarctation of aorta	6,555	1	1.53	–	–	Källén and Otterblad Olausson, 2007
SSRIs	Esophageal atresia	6,555	3	4.58	0.11	0.19	Källén and Otterblad Olausson, 2007
SSRIs	Small intestinal atresia	6,555	4	6.10	<0.001	0.005	Källén and Otterblad Olausson, 2007

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
SSRIs	Anorectal malformation	6,555	2	3 05	0.65	0 78	Kàllén and Otterblad Olausson, 2007
SSRIs	Diaphragmatic hernia	6,555	2	3.05	0.53	0.68	Kallén and Otterblad Olausson, 2007
SSRIs	Gastroschisis	6,555	2	3.05	0.47	0.54	Kàllén and Otterblad Olausson, 2007
SSRIs	Omphalocele	6,555	1	1 53	–	–	Kallén and Otterblad Olausson, 2007
SSRIs	Hypospadias	3,343	26	77.77	<0.001	0.17	Kàllén and Otterblad Olausson, 2007
SSRIs	Limb reduction	6,555	2	3.05	0 74	0.41	Kallén and Otterblad Olausson, 2007
SSRIs	Craniosynostosis	6,555	6	9.15	<0.001	0 03	Kàllén and Otterblad Olausson, 2007
Citalopram	Hypoplastic left heart	387	1	25.84	–	–	Ericson *et al*, 1999; Heikkinen *et al.*, 2002
Citalopram	Cleft lip ± cleft palate	387	1	25.84	–	–	Ericson *et al*, 1999; Heikkinen *et al.*, 2002
Citalopram	Duodenal atresia	387	1	25.84	–	–	Ericson *et al*, 1999; Heikkinen *et al*, 2002
Citalopram	Hypospadias	197	2	101.52	0 04	0.44	Ericson *et al*, 1999, Heikkinen *et al*, 2002
Escitalopram	N/A	7	0	–	–	–	Wichman *et al*, 2009
Fluoxetine	Glaucoma	1,519	1	6.58	–	–	Reis and Kallén, 2010
Fluoxetine	Ventricular septal defect	1,653	9	54.45	0 03	0.02	Wichman *et al*, 2009; Reis and Kallén, 2010
Fluoxetine	Atrial septal defect	1,519	6	39 50	0.08	<0.001	Reis and Kallén, 2010
Fluoxetine	Tricuspid atresia/stenosis	1,519	1	6.58	–	–	Reis and Kállén, 2010
Fluoxetine	Pulmonary valve stenosis	1,519	1	6 58	–	–	Reis and Kállén, 2010
Fluoxetine	Aortic valve atresia/stenosis	1,519	1	6 58	–	–	Reis and Kallén, 2010
Fluoxetine	Coarctation of aorta	1,519	1	6 58	–	–	Reis and Kallén, 2010
Fluoxetine	TAPVR	1,519	1	6 58	–	–	Reis and Kallén, 2010
Fluoxetine	Cleft lip ± cleft palate	1,519	2	13 17	0 44	0 65	Reis and Kallén, 2010
Fluoxetine	Cleft palate	1,519	1	6.58	–	–	Reis and Kallén, 2010
Fluoxetine	Esophageal atresia	1,519	2	13 17	0 001	0.004	Reis and Kállén, 2010
Fluoxetine	Gastroschisis	1,519	2	13 17	0 001	0 002	Reis and Kallén, 2010
Fluoxetine	Hypospadias	775	5	64 52	0 04	0 85	Reis and Kallén, 2010
Fluoxetine	Polydactyly	1,519	4	26.33	0 006	0 29	Reis and Kallén, 2010
Fluoxetine	Syndactyly	1,519	3	19 75	0 01	–	Reis and Kallén, 2010
Fluoxetine	Craniosynostosis	1,519	1	6 58	–	–	Reis and Kallén, 2010
Paroxetine	Spina bifida	1,562	2	12.80	0 003	0 24	Bérard *et al*, 2007, Cole *et al*, 2007
Paroxetine	Transposition of great vessels	1,020	1	9 80	–	–	Cole *et al*, 2007

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Paroxetine	Ventricular septal defect	2,339	19	81.23	<0.001	<0.001	Cole *et al.*, 2007; Wichman *et al.*, 2009; Reis and Källén, 2010
Paroxetine	Atrial septal defect	2,228	8	35.91	0.08	<0.001	Cole *et al.*, 2007; Reis and Källén, 2010
Paroxetine	Pulmonary valve stenosis	1,020	4	39.22	<0.001	<0.001	Cole *et al.*, 2007
Paroxetine	Aortic valve stenosis/atresia	1,020	1	9.80	–	–	Cole *et al.*, 2007
Paroxetine	Coarctation of aorta	1,020	1	9.80	–	–	Cole *et al.*, 2007
Paroxetine	Cleft lip ± cleft palate	1,020	2	19.61	0.17	0.30	Cole *et al.*, 2007
Paroxetine	Cleft palate	1,020	1	9.80	–	–	Cole *et al.*, 2007
Paroxetine	Anorectal malformation	1,020	2	19.61	<0.001	0.009	Cole *et al.*, 2007
Paroxetine	Omphalocele	1,020	1	9.80	–	–	Cole *et al.*, 2007
Paroxetine	Hypospadias	1,167	12	102.83	<0.001	0.05	Cole *et al.*, 2007; Reis and Källén, 2010
Sertraline	Tetralogy of Fallot	1,371	1	7.29	–	–	Källén and Otterblad Olausson, 2006
Sertraline	Hypospadias	1,681	8	47.59	0.08	0.53	Reis and Källén, 2010
Statins	Ventricular septal defect	61	1	163.93	–	–	Ofori *et al.*, 2007
Statins	Atrial septal defect	61	1	163.93	–	–	Ofori *et al.*, 2007
Sulfasalazine	N/A	40	0	–	–	–	Willoughby and Truelove, 1980
Sumatriptan	Spina bifida	725	1	13.79	–	–	O'Quinn *et al.*, 1999; Källén and Lygner, 2001
Sumatriptan	Ventricular septal defect	725	4	55.17	0.13	0.12	O'Quinn *et al.*, 1999; Källén and Lygner, 2001
Sumatriptan	Atrial septal defect	725	2	27.59	0.62	0.02	O'Quinn *et al.*, 1999; Källén and Lygner, 2001
Sumatriptan	Hypospadias	370	1	27.03	–	–	O'Quinn *et al.*, 1999; Källén and Lygner, 2001
Sumatriptan	Polydactyly	725	2	27.59	0.04	0.41	O'Quinn *et al.*, 1999; Källén and Lygner, 2001
Sumatriptan	Craniosynostosis	725	1	13.79	–	–	O'Quinn *et al.*, 1999; Källén and Lygner, 2001
Tetracycline	Hypospadias	174	5	287.36	<0.001	<0.001	Heinonen *et al.*, 1977
Thalidomide	Duodenal atresia	5	1	2,000.00	–	–	Kajii *et al.*, 1973
Thalidomide	Limb reduction	5	1	2,000.00	–	–	Kajii *et al.*, 1973
Topiramate	Ventricular septal defect	7	1	1,428.57	–	–	Westin *et al.*, 2009
Tretinoin	Hypospadias	108	1	92.59	–	–	Jick *et al.*, 1993

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Valproic acid	Spina bifida	224	3	133 93	<0 001	<0 001	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Cataract	224	1	44 64	–	–	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Ventricular septal defect	224	2	89 29	0 07	0 19	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juárez-Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Atrial septal defect	224	5	223 21	<0 001	<0 001	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguín *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Tetralogy of Fallot	224	1	44 64	–	–	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Coarctation of aorta	224	3	133 93	<0 001	<0 001	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010

Appendix 3.2 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Valproic acid	Cleft palate	224	1	44 64	–	–	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Diaphragmatic hernia	492	1	20 33	–	–	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Wide *et al*, 2004, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Hypospadias	248	15	604 84	<0 001	<0 001	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Wide *et al*, 2004, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Limb reduction	224	2	89 29	<0 001	<0 001	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Valproic acid	Craniosynostosis	492	2	40 65	<0 001	<0 001	Jager-Roman *et al*, 1986, Bertollini *et al*, 1987, Kaneko *et al*, 1988, Thisted and Ebbesen, 1993, Canger *et al*, 1999, Holmes *et al*, 2001, Wide *et al*, 2004, Meador *et al*, 2006, Juarez-Olguin *et al*, 2008, Mawer *et al*, 2010
Zidovudine	Cleft lip ± cleft palate	49	1	204 08	–	–	Kumar *et al*, 1994
Zidovudine	Hypospadias	334	6	179 64	<0 001	0 004	Kumar *et al*, 1994, Watts *et al*, 2007
Zidovudine	Polydactyly	49	1	204 08	–	–	Kumar *et al*, 1994

17OHP, 17-hydroxyprogesterone, ACE, angiotensin-converting enzyme, AT II, angiotensin II, Ca-channel block, calcium-channel blocker, DMPA, depot medroxyprogesterone acetate, hCG, human chorionic gonadotropin, horm pregn test, hormonal pregnancy test, NSAID, non-steroidal anti-inflammatory drug, OC, oral contraceptive, SSRI, selective serotonin reuptake inhibitor, TAPVR, total anomalous pulmonary venous return

Appendix 3.3 Results of the cohort studies from voluntary reporting systems.

| Drug | Birth defect | No. of infants | | Prevalence | P-value | | Reference |
		Exposed	Affected		Europe	U.S.	
Acetaminophen	N/A	66	0	–	–	–	Riggs *et al.*, 1989; McElhatton *et al.*, 1997
Acetazolamide	N/A	1	0	–	–	–	Tomson *et al.*, 2011
Amitriptyline	Ventricular septal defect	89	1	112.36	–	–	McElhatton *et al.*, 1996
Antihypertensive	Ventricular septal defect	400	1	25.00	–	–	Diav-Citrin *et al*, 2011
Antihypertensive	Anorectal malformation	400	1	25.00	–	–	Diav-Citrin *et al.*, 2011
Antihypertensive	Post. urethral valve/prune belly	400	1	25.00	–	–	Diav-Citrin *et al.*, 2011
Antihypertensive	Hypospadias	204	1	49.02	–	–	Diav-Citrin *et al.*, 2011
Antihypertensive	Limb reduction	400	1	25.00	–	–	Diav-Citrin *et al.*, 2011
Antihypertensive	Syndactyly	400	1	25.00	–	–	Diav-Citrin *et al.*, 2011
ACEi/AT II blocker	Anorectal malformation	188	1	53.19	–	–	Diav-Citrin *et al.*, 2011
ACEi/AT II blocker	Hypospadias	96	1	104.17	–	–	Diav-Citrin *et al.*, 2011
ACE inhibitors	N/A	8	0	–	–	–	Bar *et al.*, 1997
Captopril	N/A	15	0	–	–	–	Kreft-Jais *et al.*, 1988
Enalapril	N/A	6	0	–	–	–	Kreft-Jais *et al.*, 1988
AT II blockers	Coarctation of aorta	30	1	333.33	–	–	Schaefer, 2003
AT II blockers	Cleft palate	30	1	333.33	–	–	Schaefer, 2003
Ca-channel block.	Ventricular septal defect	293	1	34.13	–	–	Magee *et al.*, 1996; Weber-Schoendorfer *et al*, 2008
Ca-channel block.	Atrial septal defect	293	1	34.13	–	–	Magee *et al.*, 1996; Weber-Schoendorfer *et al.*, 2008
Ca-channel block.	Cleft palate	293	1	34.13	–	–	Magee *et al.*, 1996; Weber-Schoendorfer *et al.*, 2008
Ca-channel block.	Hypospadias	149	1	67.11	–	–	Magee *et al.*, 1996; Weber-Schoendorfer *et al.*, 2008
Ca-channel block.	Limb reduction	293	1	34.13	–	–	Magee *et al.*, 1996; Weber-Schoendorfer *et al.*, 2008
Ca-channel block.	Polydactyly	293	1	34.13	–	–	Magee *et al.*, 1996; Weber-Schoendorfer *et al.*, 2008
Amlodipine	N/A	31	0	–	–	–	Weber-Schoendorfer *et al.*, 2008
Diltiazem	N/A	29	0	–	–	–	Weber-Schoendorfer *et al.*, 2008
Nifedipine	Atrial septum defect	51	1	196.08	–	–	Weber-Schoendorfer *et al.*, 2008

Appendix 3.3 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Verapamil	Ventricular septal defect	55	1	181.82	–	–	Weber-Schoendorfer et al, 2008
Benzodiazepines	Ventricular septal defect	459	1	21 79	–	–	Pastuszak et al, 1996; Ornoy et al, 1998
Benzodiazepines	Pulmonary valve stenosis	459	1	21.79	–	–	Pastuszak et al, 1996, Ornoy et al, 1998
Benzodiazepines	Esophageal atresia	459	1	21 79	–	–	Pastuszak et al, 1996; Ornoy et al, 1998
Benzodiazepines	Polydactyly	459	2	43.57	0 004	0 13	Pastuszak et al, 1996, Ornoy et al, 1998
Alprazolam	Atrial septal defect	276	1	36.23	–	–	St Clair and Schirmer, 1992
Alprazolam	Cleft palate	276	2	72 46	<0 001	<0 001	St Clair and Schirmer, 1992
Alprazolam	Hypospadias	141	1	70.92	–	–	St. Clair and Schirmer, 1992
Clobazam	N/A	9	0	–	–	–	Tomson et al, 2011
Clonazepam	N/A	9	0	–	–	–	Morrow et al, 2006
Bromocriptine	Cleft palate	375	1	26 67	–	–	Griffith et al, 1978
Bromocriptine	Syndactyly	375	2	53.33	<0.001	–	Griffith et al, 1978
Cabergoline	N/A	49	0	–	–	–	Ricci et al, 2002
Carbamazepine	Spina bifida	281	1	35 59	–	–	Jones et al, 1989; Diav-Citrin et al, 2001, Vajda et al, 2006
Carbamazepine	Ventricular septal defect	281	2	71.17	0.14	0.34	Jones et al, 1989, Diav-Citrin et al, 2001; Vajda et al, 2006
Carbamazepine	Atrial septal defect	281	1	35 59	–	–	Jones et al, 1989, Diav-Citrin et al., 2001; Vajda et al, 2006
Carbamazepine	Cleft palate	281	1	35 59	–	–	Jones et al, 1989; Diav-Citrin et al., 2001; Vajda et al., 2006
Carbamazepine	Hypospadias	779	9	115.53	<0 001	0 04	Jones et al, 1989, Diav-Citrin et al, 2001; Tomson et al, 2011
Carbamazepine	Polydactyly	1,528	2	13 09	0 39	0 81	Jones et al, 1989, Diav-Citrin et al, 2001; Tomson et al, 2011
Carbamazepine	Craniosynostosis	281	1	35 59	–	–	Jones et al, 1989, Diav-Citrin et al, 2001, Vajda et al, 2006
Cisapride	Pulmonary valve stenosis	88	1	113.64	–	–	Bailey et al, 1997
Cisapride	Duodenal atresia/stenosis	88	1	113 64	–	–	Bailey et al, 1997
Cisapride	Limb reduction	88	1	113 64	–	–	Bailey et al, 1997

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Clomipramine	N/A	87	0	–	–	–	McElhatton et al, 1996
Diclofenac	Cataract	123	1	81 30	–	–	Cassina et al, 2010
Diclofenac	Ventricular septal defect	123	1	81 30	–	–	Cassina et al, 2010
Diclofenac	Pulmonary valve stenosis	123	1	81 30	–	–	Cassina et al, 2010
Diclofenac	Cleft lip ± cleft palate	123	1	81 30	–	–	Cassina et al, 2010
Diclofenac	Gastroschisis	123	1	81 30	–	–	Cassina et al, 2010
Diclofenac	Syndactyly	123	1	81 30	–	–	Cassina et al, 2010
Doxepin	N/A	8	0	–	–	–	McElhatton et al, 1996
Ibuprofen	N/A	22	0	–	–	–	Barry et al, 1984
Imipramine	Omphalocele	27	1	370 37	–	–	McElhatton et al, 1996
Imipramine	Polydactyly	27	1	370 37	–	–	McElhatton et al, 1996
Isotretinoin	Anotia	60	2	333 33	<0 001	–	Honein et al, 2001, Garcia-Bournissen et al, 2008, Autret-Leca et al, 2010, Schaefer et al, 2010
Isotretinoin	Ventricular septal defect	60	4	666 67	<0 001	<0 001	Honein et al, 2001, Garcia-Bournissen et al, 2008, Autret-Leca et al, 2010, Schaefer et al, 2010
Isotretinoin	Aortic valve atresia/stenosis	46	1	217 39	–	–	Honein et al, 2001, Autret-Leca et al, 2010, Schaefer et al, 2010
Lamotrigine	Anencephaly	2,299	1	4 35	–	–	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Lamotrigine	Holoprosencephaly	2,299	1	4 35	–	–	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Lamotrigine	Transposition of great vessels	1,615	1	18 58	–	–	Vajda et al, 2006, Cunnington et al, 2011
Lamotrigine	Ventricular septal defect	1,615	3	18 58	0 54	0 56	Vajda et al, 2006, Cunnington et al, 2011
Lamotrigine	Tetralogy of Fallot	1,615	1	6 19	–	–	Vajda et al, 2006, Cunnington et al, 2011
Lamotrigine	Pulmonary valve stenosis	1,615	1	6 19	–	–	Vajda et al, 2006, Cunnington et al, 2011
Lamotrigine	Hypoplastic left heart	1,615	1	6 19	–	–	Vajda et al, 2006, Cunnington et al, 2011
Lamotrigine	Choanal atresia	2,299	1	4 35	–	–	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011

Appendix 3.3 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Lamotrigine	Cleft lip ± cleft palate	2,299	3	13 05	0 35	0 59	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Lamotrigine	Cleft palate	2,299	4	17 40	0 007	0 01	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Lamotrigine	Anorectal malformation	2,299	1	4 35	–	–	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Lamotrigine	Diaphragmatic hernia	1,615	2	12 38	0 003	0 007	Vajda et al, 2006, Cunnington et al, 2011
Lamotrigine	Post urethral valve/prune belly	2,299	2	8 70	<0 001	<0 001	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Lamotrigine	Hypospadias	1,445	6	41 52	0 25	0 38	Cunnington et al, 2011, Tomson et al, 2011
Lamotrigine	Limb reduction	2,299	2	8 70	0 23	0 50	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Lamotrigine	Polydactyly	2,834	2	7 06	0 97	0 25	Cunnington et al, 2011, Tomson et al, 2011
Lamotrigine	Craniosynostosis	2,299	1	4 35	–	–	Vajda et al, 2006, Holmes et al, 2008, Cunnington et al, 2011
Maprotiline	N/A	77	0	–	–	–	McElhatton et al, 1996
Methotrexate	N/A	19	0	–	–	–	Lewden et al, 2004
Metoclopramide	Ventricular septal defect	158	2	126 58	0 01	0 01	Berkovitch et al, 2002
Metoclopramide	Hypospadias	81	2	246 91	<0 001	0 03	Berkovitch et al, 2002
Metronidazole	Ventricular septal defect	131	1	76 34	–	–	Diav-Citrin et al, 2001
Mianserin	N/A	37	0	–	–	–	McElhatton et al, 1996
Mirtazapine	N/A	75	0	–	–	–	Yaris et al, 2004, Einarson et al, 2009
Misoprostol	N/A	67	0	–	–	–	Schuler et al, 1999
Naratriptan	Ventricular septal defect	46	1	217 39	–	–	Kendle International Inc, 2011
Nortriptyline	N/A	4	0	–	–	–	McElhatton et al, 1996
Olanzapine	N/A	18	0	–	–	–	Goldstein et al, 2000
Ondansetron	Pulmonary valve stenosis	169	1	59 17	–	–	Einarson et al, 2004
Ondansetron	Duodenal atresia	169	1	59 17	–	–	Einarson et al, 2004
Ondansetron	Hypospadias	86	3	348 84	<0 001	<0 001	Einarson et al, 2004
Oral contraceptives	N/A	99	0	–	–	–	Ahn et al, 2008
Phenobarbital	Ventricular septal defect	77	1	129 87	–	–	Holmes et al, 2004

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Phenobarbital	Tetralogy of Fallot	77	1	129.87	–	–	Holmes *et al*, 2004
Phenobarbital	Pulmonary valve atresia	77	1	129.87	–	–	Holmes *et al*, 2004
Phenobarbital	Coarctation of aorta	77	1	129.87	–	–	Holmes *et al.*, 2004
Phenobarbital	Cleft lip ± cleft palate	77	1	129.87	–	–	Holmes *et al*, 2004
Phenobarbital	Hypospadias	150	1	66 67	–	–	Holmes *et al*, 2004; Tomson *et al.*, 2011
Phenobarbital	Polydactyly	294	2	68 08	<0.001	0.02	Holmes *et al*, 2004; Tomson *et al*, 2011
Phenytoin	Anencephaly	17	1	588.24	–	–	Vajda *et al*, 2006
Promethazine	N/A	13	0	–	–	–	Diav-Citrin *et al*, 2003
Pyrimethamine	Pulmonary valve stenosis	149	1	67.11	–	–	Phillips-Howard *et al*, 1998
Rizatriptan	N/A	23	0	–	–	–	Fiore *et al*, 2005
SSRIs	Ventricular septal defect	353	1	28.33	–	–	Einarson *et al.*, 2001, Sivojelezova *et al.*, 2005
SSRIs	Atrial septal defect	353	1	28.33	–	–	Einarson *et al*, 2001; Sivojelezova *et al*, 2005
Citalopram	Atrial septal defect	184	1	54.35	–	–	Einarson *et al*, 2009
Citalopram	Hypospadias	94	1	106.38	–	–	Einarson *et al*, 2009
Escitalopram	N/A	21	0	–	–	–	Einarson *et al*, 2009
Fluoxetine	Transposition of great vessels	643	1	15.55	–	–	Pastuszak *et al*, 1993; Chambers *et al*, 1996; McElhatton *et al*, 1996, Diav-Citrin *et al*, 2008; Einarson *et al.*, 2009
Fluoxetine	Ventricular septal defect	643	8	124.42	<0.001	<0.001	Pastuszak *et al*, 1993; Chambers *et al.*, 1996; McElhatton *et al*, 1996, Diav-Citrin *et al*, 2008; Einarson *et al*, 2009
Fluoxetine	Atrial septal defect	643	3	46.66	0 12	<0.001	Pastuszak *et al*, 1993, Chambers *et al.*, 1996; McElhatton *et al*, 1996, Diav-Citrin *et al*, 2008; Einarson *et al*, 2009
Fluoxetine	Ebstein's anomaly	643	1	15 55	–	–	Pastuszak *et al*, 1993, Chambers *et al*, 1996, McElhatton *et al*, 1996, Diav-Citrin *et al*, 2008, Einarson *et al*, 2009
Fluoxetine	Pulmonary valve stenosis	643	2	31 10	<0 001	<0 001	Pastuszak *et al*, 1993, Chambers *et al*, 1996, McElhatton *et al*, 1996, Diav-Citrin *et al*, 2008; Einarson *et al*, 2009

Appendix 3.3 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Fluoxetine	Aortic valve atresia/stenosis	643	1	15 55	–	–	Pastuszak et al., 1993, Chambers et al, 1996; McElhatton et al., 1996, Diav-Citrin et al., 2008; Einarson et al, 2009
Fluoxetine	Small intestinal atresia	643	1	15 55	–	–	Pastuszak et al., 1993, Chambers et al, 1996; McElhatton et al, 1996; Diav-Citrin et al, 2008; Einarson et al, 2009
Fluoxetine	Hypospadias	328	3	91 46	0 02	0 45	Pastuszak et al., 1993, Chambers et al., 1996; McElhatton et al, 1996; Diav-Citrin et al, 2008; Einarson et al., 2009
Fluoxetine	Craniosynostosis	643	3	46.66	<0.001	<0 001	Pastuszak et al, 1993; Chambers et al, 1996, McElhatton et al, 1996, Diav-Citrin et al., 2008; Einarson et al, 2009
Fluvoxamine	Atrial septal defect	102	1	98 04	–	–	McElhatton et al, 1996, Einarson et al, 2009
Paroxetine	Ventricular septal defect	499	4	80 16	0 02	0 02	McElhatton et al, 1996; Diav-Citrin et al, 2008, Einarson et al., 2009
Paroxetine	Atrial septal defect	499	2	40.08	0.30	0.002	McElhatton et al, 1996, Diav-Citrin et al, 2008, Einarson et al, 2009
Paroxetine	Pulmonary valve stenosis	499	1	20 04	–	–	McElhatton et al, 1996; Diav-Citrin et al, 2008, Einarson et al, 2009
Paroxetine	Hypoplastic left heart	499	1	20 04	–	–	McElhatton et al, 1996; Diav-Citrin et al., 2008; Einarson et al., 2009
Paroxetine	Coarctation of aorta	499	1	20 04	–	–	McElhatton et al, 1996; Diav-Citrin et al, 2008; Einarson et al, 2009
Paroxetine	Cleft lip ± cleft palate	499	1	20 04	–	–	McElhatton et al, 1996; Diav-Citrin et al, 2008; Einarson et al, 2009
Paroxetine	Omphalocele	499	1	20 04	–	–	McElhatton et al, 1996, Diav-Citrin et al, 2008; Einarson et al, 2009
Paroxetine	Post urethral valve/prune belly	499	1	20 04	–	–	McElhatton et al, 1996, Diav-Citrin et al, 2008, Einarson et al, 2009
Paroxetine	Craniosynostosis	499	1	20 04	–	–	McElhatton et al., 1996, Diav-Citrin et al, 2008, Einarson et al., 2009

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Sertraline	N/A	61	0	–	–	–	Einarson *et al*, 2009
Statins	Spina bifida	264	2	75 76	<0 001	<0 001	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Holoprosencephaly	264	1	37 88	–	–	Edison and Muenke *et al*, 2004 & 2005, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Ventricular septal defect	264	1	37 88	–	–	Edison and Muenke *et al*, 2004 & 2005, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Atrial septal defect	264	1	37 88	–	–	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Cleft lip ± cleft palate	264	3	113 64	<0 001	<0 001	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Cleft palate	264	1	37 88	–	–	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Duodenal atresia/stenosis	264	2	75 76	<0 001	<0 001	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Anorectal malformation	264	1	37 88	–	–	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Hypospadias	135	2	148 15	0 005	0 18	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Limb reduction	264	5	189 39	<0 001	<0 001	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Statins	Polydactyly	264	2	75 76	<0 001	0 01	Edison and Muenke *et al*, 2004, Pollack *et al*, 2005, Taguchi *et al*, 2008
Sumatriptan	Ventricular septal defect	544	4	73 53	0 03	0 03	Shuhaiber *et al*, 1998, Kendle International Inc, 2011
Sumatriptan	Cleft lip ± cleft palate	544	1	18 38	–	–	Shuhaiber *et al*, 1998, Kendle International Inc, 2011
Sumatriptan	Anorectal malformation	544	1	18 38	–	–	Shuhaiber *et al*, 1998, Kendle International Inc, 2011
Sumatriptan	Biliary atresia	544	1	18 38	–	–	Shuhaiber *et al*, 1998, Kendle International Inc, 2011

Appendix 3.3 (Continued)

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Sumatriptan	Diaphragmatic hernia	544	1	18 38	–	–	Shuhaiber *et al*, 1998, Kendle International Inc, 2011
Sumatriptan	Hypospadias	277	1	36 10	–	–	Shuhaiber *et al*, 1998, Kendle International Inc, 2011
Sumatriptan	Polydactyly	544	1	18 38	–	–	Shuhaiber *et al*, 1998, Kendle International Inc, 2011
Sumatriptan	Craniosynostosis	544	1	18 38	–	–	Shuhaiber *et al*, 1998, Kendle International Inc, 2011
Tiaprofenic acid	Ventricular septal defect	7	1	1,428 57	–	–	Pastuszak *et al*, 1993
Topiramate	Cleft lip ± cleft palate	103	2	194 17	<0 001	<0 001	Hunt *et al*, 2008, Ornoy *et al*, 2008
Topiramate	Hypospadias	53	1	188 68	–	–	Hunt *et al*, 2008, Ornoy *et al*, 2008
Trazodone	N/A	27	0	–	–	–	McElhatton *et al*, 1996, Einarson *et al*, 2009
Tretinoin	Diaphragmatic hernia	177	1	56 50	–	–	Shapiro *et al*, 1997, Loureiro *et al*, 2005
Valproic acid	Spina bifida	444	4	90 09	<0 001	<0 001	Lindhout and Schmidt, 1986, Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Ventricular septal defect	325	3	92 31	0 02	0 10	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Atrial septal defect	325	8	246 15	<0 001	<0 001	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Tetralogy of Fallot	325	1	30 77	–	–	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Tricuspid atresia/stenosis	325	1	30 77	–	–	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Pulmonary valve stenosis	325	1	30 77	–	–	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Pulmonary valve atresia	325	2	61 54	<0 001	<0 001	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Cleft palate	325	3	92 31	<0 001	<0 001	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Hypospadias	625	22	352 00	<0 001	<0 001	Wyszynski *et al*, 2005, Diav-Citrin *et al*, 2008, Tomson *et al*, 2011

Drug	Birth defect	No. of infants		Prevalence	P-value		Reference
		Exposed	Affected		Europe	U.S.	
Valproic acid	Polydactyly	1,226	6	48 94	<0 001	0 003	Wyszynski *et al*, 2005, Diav-Citrin *et al*, 2008, Tomson *et al*, 2011
Valproic acid	Craniosynostosis	325	2	61 54	<0 001	<0 001	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Valproic acid	Situs inversus	325	1	30 77	–	–	Wyszynski *et al*, 2005, Vajda *et al*, 2006, Diav-Citrin *et al*, 2008
Vitamin A	Pulmonary valve stenosis	311	1	32 15	–	–	Mastroiacovo *et al*, 1999
Vitamin A	Anorectal malformation	311	1	32 15	–	–	Mastroiacovo *et al*, 1999
Zidovudine	N/A	58	0	–	–	–	Sperling *et al*, 1992, Anonymous, 1994

ACE, angiotensin-converting enzyme, ACEi, angiotensin-converting enzyme inhibitor, AT II, angiotensin II, Ca-channel block , calcium-channel blocker, SSRI, selective serotonin-reuptake inhibitor

Appendix 3.4 Results of the case-control studies with population controls

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Acetaminophen	Anencephaly	98	196	1,956	4,143	1 1 (0.8-1.5)	1.2 (0.9-1 7)	Feldkamp *et al*, 2010
Acetaminophen	Spina bifida	207	435	1,956	4,143	1 0 (0.8-1 2)	1 1 (0.9-1 3)	Feldkamp *et al*, 2010
Acetaminophen	Anophthalmia	49	101	1,956	4,143	1 1 (0.8-1 6)	1.0 (0.7-1 5)	Feldkamp *et al*, 2010
Acetaminophen	Cataract	56	131	1,956	4,143	0 8 (0 6-1 2)	0 8 (0.6-1.2)	Feldkamp *et al*, 2010
Acetaminophen	Anotia	98	244	1,956	4,143	0 8 (0.6-1 0)	1 0 (0.8-1.3)	Feldkamp *et al*, 2010
Acetaminophen	Transposition of great vessels	171	342	1,956	4,143	1 1 (0.9-1.4)	1 1 (0 9-1 4)	Feldkamp *et al*, 2010
Acetaminophen	Ventricular septal defect	488	998	1,956	4,143	1.1 (0.9-1 2)	NR	Feldkamp *et al.*, 2010
Acetaminophen	Atrial septal defect	604	1,199	1,956	4,143	1 1 (1.0-1.3)	1 1 (0 9-1.2)	Feldkamp *et al*, 2010
Acetaminophen	Atrioventricular septal defect	49	104	1,956	4,143	1 0 (0 7-1.5)	0 9 (0 6-1 4)	Feldkamp *et al*, 2010
Acetaminophen	Tetralogy of Fallot	191	395	1,956	4,143	1.0 (0.9-1.3)	1 1 (0 8-1 3)	Feldkamp *et al*, 2010
Acetaminophen	Pulmonary valve stenosis	267	508	1,956	4,143	1 2 (1 0-1 5)	1.1 (0.9-1.4)	Feldkamp *et al.*, 2010
Acetaminophen	Aortic stenosis	73	149	1,956	4,143	1 1 (0 8-1.5)	0 9 (0 7-1.3)	Feldkamp *et al*, 2010
Acetaminophen	Hypoplastic left heart	101	206	1,956	4,143	1 1 (0 8-1.4)	1.0 (0 7-1 3)	Feldkamp *et al.*, 2010
Acetaminophen	Coarctation of aorta	188	396	1,956	4,143	1 0 (0 8-1.2)	0 9 (0 7-1 1)	Feldkamp *et al*, 2010
Acetaminophen	TAPVR	52	103	1,956	4,143	1 1 (0 8-1 7)	1 2 (0 8-1.7)	Feldkamp *et al*, 2010
Acetaminophen	Cleft lip ± cleft palate	519	1,110	1,956	4,143	1 0 (0 9-1 1)	1 0 (0 8-1 1)	Feldkamp *et al.*, 2010
Acetaminophen	Cleft lip ± cleft palate	37	656	36,564	575,829	0.9 (0 6-1.3)	NR	Kalléen, 2003
Acetaminophen	Cleft lip ± cleft palate	6	1,374	50	38,151	3.3 (1 4-7 8)	2 1 (0 9-5 0)	Puhó *et al*, 2007
Acetaminophen	Cleft palate	296	570	1,956	4,143	1 2 (1.0-1.4)	1.1 (0 9-1.4)	Feldkamp *et al*, 2010
Acetaminophen	Cleft palate	25	388	36,564	575,829	1.0 (0 7-1 5)	NR	Kalléen, 2003
Acetaminophen	Cleft palate	3	601	49	38,151	3.9 (1 2-12.6)	3.7 (1 1-12 0)	Puhó *et al*, 2007
Acetaminophen	Esophageal atresia	124	265	1,956	4,143	1 0 (0.8-1 3)	1.0 (0 7-1.3)	Feldkamp *et al*, 2010
Acetaminophen	Intestinal atresia	82	174	1,956	4,143	1.0 (0 7-1 4)	1 2 (0 9-1 7)	Feldkamp *et al*, 2010
Acetaminophen	Small intestinal atresia	73	127	202	416	1.4 (1.0-2.1)	NR	Werler *et al*, 2003
Acetaminophen	Anorectal malformation	155	365	1,956	4,143	0 8 (0 7-1 0)	0.9 (0.7-1.1)	Feldkamp *et al*, 2010
Acetaminophen	Diaphragmatic hernia	153	300	1,956	4,143	1.2 (0.9-1 5)	1 2 (0 9-1 5)	Feldkamp *et al*, 2010
Acetaminophen	Gastroschisis	120	205	202	416	1 5 (1.1-2 1)	NR	Werler *et al*, 2003
Acetaminophen	Gastroschisis	28	110	56	220	1 0 (0.6-1 7)	NR	Torfs *et al*, 1996
Acetaminophen	Gastroschisis	212	467	1,956	4,143	0 9 (0 8-1.1)	1 0 (0 8-1 3)	Feldkamp *et al*, 2010
Acetaminophen	Omphalocele	75	169	1,956	4,143	0 9 (0 7-1 2)	0.9 (0.7-1.3)	Feldkamp *et al*, 2010
Acetaminophen	Hypospadias	333	758	990	2,094	0 9 (0 7-1.0)	0 9 (0 7-1.1)	Feldkamp *et al*, 2010

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Acetaminophen	Limb reduction	256	528	1,956	4,143	1.1 (0 9-1.3)	NR	Feldkamp *et al*, 2010
Acetaminophen	Craniosynostosis	240	479	1,956	4,143	1.1 (0.9-1 4)	1.0 (0.8-1.2)	Feldkamp *et al.*, 2010
Antihypertensive	Transposition of great vessels	3	328	30	4,796	1.5 (0 4-4 8)	1.5 (0 3-4.8)	Caton *et al*, 2009
Antihypertensive	Single ventricle	0	156	30	4,796	–	–	Caton *et al*, 2009
Antihypertensive	Perimembranous VSD	10	878	30	4,796	1 8 (0.9-3 8)	1.7 (0 8-3.5)	Caton *et al*, 2009
Antihypertensive	ASD secundum type	19	1,137	30	4,796	2 7 (1 5-3 8)	2.4 (1 3-4.4)	Caton *et al.*, 2009
Antihypertensive	Atrioventricular septal defect	2	126	30	4,796	2.6 (0.6-10.8)	2.6 (0.3-10.3)	Caton *et al*, 2009
Antihypertensive	Tetralogy of Fallot	4	466	30	4,796	1 4 (0 5-3 9)	1.4 (0 4-4.0)	Caton *et al*, 2009
Antihypertensive	Pulmonary valve stenosis	11	534	30	4,796	3 3 (1.7-6 7)	2.6 (1 3-5 4)	Caton *et al*, 2009
Antihypertensive	Aortic stenosis	0	160	30	4,796	–		Caton *et al*, 2009
Antihypertensive	Hypoplastic left heart	1	233	30	4,796	0 7 (0 1-5 0)	0 7 (0 1-4.1)	Caton *et al*, 2009
Antihypertensive	Coarctation of aorta	8	406	30	4,796	3 2 (1.5-7 0)	3 0 (1 3-6.6)	Caton *et al*, 2009
Antihypertensive	TAPVR	1	118	30	4,796	1 4 (0.2-10.0)	1.3 (0 1-8 0)	Caton *et al*, 2009
Antihypertensive	Cleft lip ± cleft palate	2	656	1,992	575,829	0 9 (0 2-3 5)	NR	Kallén, 2003
Antihypertensive	Cleft palate	2	388	1,992	575,829	1 5 (0.4-6 0)	NR	Kallén, 2003
Antihypertensive	Gastroschisis	5	514	22	3,277	0.6 (0.1-2 5)	2.6 (0 9-8.0)	Werler *et al*, 2009b
Antihypertensive	Hypospadias	15	758	24	2,058	1 9 (1.0-3.6)	1 4 (0.7-2.9)	Caton *et al.*, 2008
ACE inhibitor	Hypospadias	1	758	5	2,058	0.5 (0.1-4.7)	NR	Caton *et al*, 2008
AT II blocker	Hypospadias	0	758	0	2,058	–	–	Caton *et al.*, 2008
Atenolol	Hypospadias	2	758	4	2,058	1 4 (0 2-7 4)	NR	Caton *et al*, 2008
Ca-channel block.	Cleft lip ± cleft palate	4	1,246	0	1,246	–	–	Sørensen *et al*, 2001
Ca-channel block.	Cleft palate	1	537	2	537	0.5 (0 0-5 5)	0 4 (0.0-4 6)	Sørensen *et al*, 2001
Ca-channel block.	Esophageal atresia	0	192	1	192	–	–	Sørensen *et al*, 2001
Ca-channel block.	Intestinal atresia	0	132	0	132	–	–	Sørensen *et al*, 2001
Ca-channel block.	Hypospadias	4	2,817	3	2,817	1 3 (0.3-6.0)	1.3 (0.3-5 9)	Sørensen *et al.*, 2001
Ca-channel block.	Hypospadias	2	758	5	2,058	1 1 (0.2-5 6)	NR	Caton *et al*, 2009
Ca-channel block.	Limb reduction	1	493	0	493	–	–	Sørensen *et al.*, 2001
Furosemide	Cleft lip ± cleft palate	1	1,368	15	38,151	1 9 (0.2-14 1)	NR	Czeizel and Rockenbauer, 1999
Furosemide	Cleft palate	0	596	15	38,151	–	–	Czeizel and Rockenbauer, 1999
Furosemide	Esophageal atresia	2	229	15	38,151	22 8 (5 2-101)	NR	Czeizel and Rockenbauer, 1999
Furosemide	Anorectal malformation	0	220	15	38,151	–	–	Czeizel and Rockenbauer, 1999

Appendix 3.4 (Continued)

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Furosemide	Limb reduction	1	545	15	38,151	4.4 (0 6-33 5)	NR	Czeizel and Rockenbauer, 1999
Methyldopa	Hypospadias	6	758	10	2,058	1.6 (0.6-4.5)	NR	Caton et al., 2008
Metoprolol	Cleft palate	4	601	120	38,151	2.1 (0.8-5.8)	2.1 (0 8-5.7)	Puhó et al., 2007
Oxprenolol	Cleft lip ± cleft palate	6	1,374	41	38,151	4 1 (1 7-9.6)	4.2 (1.8-10.0)	Puhó et al., 2007
Oxprenolol	Cleft palate	3	601	50	38,151	3.8 (1.2-12 3)	3.6 (1 1-11.7)	Puhó et al., 2007
Propranolol	Cleft lip ± cleft palate	2	578	0	578	–	–	Czeizel, 1989
Propranolol	Hypospadias	1	1,191	0	1,191	–	–	Czeizel, 1989
Benzodiazepines	Cleft lip ± cleft palate	1	656	1,008	575,829	0.9 (0.1-6.2)	NR	Kállén, 2003
Benzodiazepines	Cleft palate	0	388	1,008	575,829		–	Kállén, 2003
Chlordiazepoxide	Cleft lip ± cleft palate	2	1,369	3	1,369	0.7 (0.1-4.0)	0.6 (0.1-3.4)	Czeizel et al., 2004
Chlordiazepoxide	Cleft lip ± cleft palate	4	630	2	630	2.0 (0.4-11.0)	NR	Czeizel, 1987
Chlordiazepoxide	Cleft palate	4	601	84	38,151	3.0 (1.1-8.3)	2.9 (1.1-7 9)	Puhó et al., 2007
Chlordiazepoxide	Cleft palate	2	179	1	179	2.0 (0.2-22.4)	NR	Czeizel, 1987
Chlordiazepoxide	Esophageal atresia	3	214	0	214	7.0 (0 4-136)	–	Czeizel et al., 2004
Chlordiazepoxide	Anorectal malformation	1	220	0	220	3.0 (0.1-73 7)	–	Czeizel et al., 2004
Chlordiazepoxide	Hypospadias	9	3,033	4	3,033	2.3 (0 7-7 3)	2 2 (0 7-6.7)	Czeizel et al., 2004
Chlordiazepoxide	Limb reduction	1	545	3	545	0.3 (0.0-3 2)	0.5 (0.0-4.5)	Czeizel et al., 2004
Diazepam	Cleft lip ± cleft palate	43	1,374	704	38,151	1.7 (1.3-2 3)	1.7 (1 3-2.3)	Puhó et al., 2007
Diazepam	Cleft lip ± cleft palate	16	630	17	630	0.9 (0.5-1.9)	NR	Czeizel, 1987
Diazepam	Cleft palate	17	601	1,077	38,151	1.0 (0.6-1 6)	1.0 (0 6-1.6)	Puhó et al., 2007
Diazepam	Cleft palate	3	179	4	179	0.7 (0.2-3.4)	NR	Czeizel, 1987
Diazepam	Esophageal atresia	NR	217	NR	217	1.1 (0.4-2 8)	–	Kjær et al., 2007
Diazepam	Intestinal atresia	NR	153	NR	153	3.2 (1.0-9 9)	–	Kjær et al., 2007
Diazepam	Anorectal malformation	NR	220	NR	220	1.6 (0.7-3 7)	–	Kjær et al., 2007
Diazepam	Hypospadias	NR	3,038	NR	3,038	0.8 (0.6-1 2)	–	Kjær et al., 2007
Diazepam	Limb reduction	NR	548	NR	548	2.6 (1.3-4.9)	–	Kjær et al., 2007
Nitrazepam	Cleft lip ± cleft palate	2	630	0	630	–	–	Czeizel, 1987
Nitrazepam	Cleft palate	0	179	1	179	–	–	Czeizel, 1987
Carbamazepine	Cleft palate	3	601	15	38,151	12.8 (3.7-44.2)	13.7 (3.9-47.5)	Puhó et al., 2007
Dextromethorphan	Cleft lip ± cleft palate	3	471	0	453	–	–	Martínez-Frías and Rodríguez-Pinilla, 2001

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Dextromethorphan	Cleft palate	4	691	1	681	4.0 (0.4-93.2)	3 3 (0.4-30.3)	Martínez-Frías and Rodríguez-Pinilla, 2001
Dextromethorphan	Small intestinal atresia	12	127	33	416	1 2 (0 6-2 4)	NR	Werler et al, 2003
Dextromethorphan	Gastroschisis	17	205	33	416	1 0 (0 6-1 9)	NR	Werler et al, 2003
Doxycycline	Cleft lip ± cleft palate	1	1,005	1	1,005	1.0 (0.1-16 0)	NR	Czeizel and Rockenbauer, 1997
Doxycycline	Esophageal atresia	0	163	0	163	–	–	Czeizel and Rockenbauer, 1997
Doxycycline	Hypospadias	3	2,237	1	2,237	3 0 (0.3-28 8)	NR	Czeizel and Rockenbauer, 1997
Ephedrine	Gastroschisis	2	381	2	4,121	10 9 (1 5-77 4)	NR	Lin et al, 2008
Epinephrine	Gastroschisis	1	381	1	4,121	10 8 (0 7-174)	NR	Lin et al, 2008
Metronidazol (oral)	Cleft lip ± cleft palate	8	940	1	940	8.1 (1.0-64.6)	8.5 (1 1-68.9)	Czeizel and Rockenbauer, 1998
Metronidazol (vag)	Cleft lip ± cleft palate	6	1,374	98	38,151	1 7 (0 7-3 9)	2 2 (0 6-8.1)	Kazy et al, 2005
Metronidazol (oral)	Cleft palate	1	435	0	435	–	–	Czeizel and Rockenbauer, 1998
Metronidazol (vag.)	Cleft palate	0	582	98	38,151	–	–	Kazy et al., 2005
Metronidazol (vag)	Esophageal atresia	4	217	98	38,151	7 3 (2 7-20 0)	7.0 (0 7-67.0)	Kazy et al, 2005
Metronidazol (oral)	Anorectal malformation	3	149	0	149	–	–	Czeizel and Rockenbauer, 1998
Metronidazol (vag.)	Anorectal malformation	0	220	98	38,151	–	–	Kazy et al., 2005
Metronidazol (oral)	Hypospadias	11	2,064	12	2,064	0.9 (0.4-2.1)	0.8 (0.4-1.8)	Czeizel and Rockenbauer, 1998
Metronidazol (oral)	Limb reduction	1	395	4	395	0 2 (0.0-2 2)	0 2 (0 0-2 1)	Czeizel and Rockenbauer, 1998
Metronidazol (vag.)	Limb reduction	2	548	98	38,151	1 4 (0.3-5 8)	1.2 (0 2-9.2)	Kazy et al, 2005
Misoprostol	Cleft lip ± cleft palate	3	134	23	4,980	4 9 (1 5-16.6)	NR	Orioli and Castilla, 2000
Misoprostol	Polydactyly	2	318	23	4,980	1.4 (0.3-5.8)	NR	Orioli and Castilla, 2000
Nitrofurantoin	Cleft lip ± cleft palate	11	1,374	186	38,151	1 6 (0.9-3 0)	1.1 (0 6-2 0)	Puhó et al., 2007
Nitrofurantoin	Cleft palate	5	601	280	38,151	1 1 (0 5-2.8)	1 0 (0.4-2 4)	Puhó et al., 2007
Nitrofurantoin	Esophageal atresia	2	192	0	192	–	–	Czeizel et al, 2001
Nitrofurantoin	Intestinal atresia	0	132	2	132	–	–	Czeizel et al, 2001
Nitrofurantoin	Anorectal malformation	2	201	1	201	2 0 (0 2-22.3)	2 8 (0.2-34 3)	Czeizel et al, 2001
Nitrofurantoin	Hypospadias	14	2,817	7	2,817	2 0 (0 8-5 0)	2 1 (0 8-5 2)	Czeizel et al, 2001
Nitrofurantoin	Limb reduction	3	493	3	493	1.0 (0.2-5.0)	0 6 (0.1-3 5)	Czeizel et al, 2001
NSAIDs	Ventricular septal defect	21	296	5	296	4.2 (1.5-14 3)	NR	Bateman et al, 2004
NSAIDs	Muscular VSD	30	164	128	677	1 0 (0 6-1 5)	1 0 (0 6-1 6)	Cleves et al., 2004
NSAIDs	Cleft lip ± cleft palate	9	656	7,684	575,829	1.0 (0.5-2.0)	NR	Kallén, 2003

Appendix 3.4 (Continued)

Drug	Birth defect	Cases Exposed	Cases Total	Controls Exposed	Controls Total	Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
NSAIDs	Cleft palate	5	388	7,684	575,829	1.0 (0.4-2.3)	NR	Källén, 2003
NSAIDs	Gastroschisis	150	514	755	3,277	1.4 (1.1-1.7)	1.4 (1.1-1.7)	Werler et al., 2009b
NSAIDs	Transverse limb deficiency	99	373	1,545	5,958	1.0 (0.8-1.3)	1.3 (1.0-1.6)	Werler et al., 2009a
Ibuprofen	Small intestinal atresia	15	127	47	416	1.1 (0.6-2.0)	NR	Werler et al., 2003
Ibuprofen	Gastroschisis	29	205	47	416	1.3 (0.8-2.1)	NR	Werler et al., 2003
Ibuprofen	Gastroschisis	6	110	3	220	4.0 (1.0-16.0)	NR	Torfs et al., 1996
Ibuprofen	Gastroschisis	131	485	1,063	4,967	1.4 (1.1-1.7)	1.6 (1.2-2.1)	Mac Bird et al., 2009
Ibuprofen	Omphalocele	41	168	1,063	4,967	1.2 (0.8-1.7)	1.2 (0.8-1.8)	Mac Bird et al., 2009
Naproxen	Cleft lip ± cleft palate	5	656	1,671	575,829	2.6 (1.1-6.4)	NR	Källén, 2003
Naproxen	Cleft palate	3	388	1,671	575,829	2.7 (0.9-8.3)	NR	Källén, 2003
Naproxen	Gastroschisis	31	485	239	4,967	1.4 (0.9-2.0)	1.0 (0.6-1.6)	Mac Bird et al., 2009
Naproxen	Omphalocele	10	168	239	4,967	1.3 (0.7-2.4)	1.0 (0.5-2.1)	Mac Bird et al., 2009
Oxytetracycline	Cleft lip ± cleft palate	0	1,247	2	1,247	–	–	Czeizel and Rockenbauer, 2000
Oxytetracycline	Cleft palate	4	601	50	38,151	5.1 (1.8-14.2)	4.3 (1.5-12.0)	Puhó et al., 2007
Oxytetracycline	Anorectal malformation	2	201	0	201	–	–	Czeizel and Rockenbauer, 2000
Oxytetracycline	Hypospadias	8	2,817	2	2,817	4.0 (0.9-18.9)	4.4 (0.9-20.7)	Czeizel and Rockenbauer, 2000
Oxytetracycline	Limb reduction	1	493	0	493	–	–	Czeizel and Rockenbauer, 2000
Phenobarbital	Cleft lip ± cleft palate	12	1,374	161	38,151	3.1 (1.2-3.7)	2.9 (1.0-8.4)	Puhó et al., 2007
Phenobarbital	Cleft palate	4	601	176	38,151	1.4 (0.5-3.9)	1.5 (0.5-4.0)	Puhó et al., 2007
Phenytoin	Cleft lip ± cleft palate	10	1,374	73	38,151	3.8 (2.0-7.4)	3.0 (1.5-5.8)	Puhó et al., 2007
Promethazine	Anencephaly	9	234	127	4,982	1.5 (0.8-3.0)	1.0 (0.5-2.3)	Gilboa et al., 2009
Promethazine	Spina bifida	20	489	127	4,982	1.6 (1.0-2.6)	1.8 (1.1-3.1)	Gilboa et al., 2009
Promethazine	Anotia/microtia	4	218	127	4,982	0.7 (0.3-2.0)	1.8 (0.6-5.2)	Gilboa et al., 2009
Promethazine	Transposition of great vessels	4	278	127	4,982	0.6 (0.2-1.5)	0.5 (0.2-1.6)	Gilboa et al., 2009
Promethazine	Perimembranous VSD	23	616	127	4,982	1.5 (0.9-2.3)	1.3 (0.8-2.1)	Gilboa et al., 2009
Promethazine	ASD secundum	21	475	127	4,982	1.8 (1.1-2.8)	1.2 (0.7-2.0)	Gilboa et al., 2009
Promethazine	Tetralogy of Fallot	15	391	127	4,982	1.5 (0.9-2.6)	1.5 (0.8-2.7)	Gilboa et al., 2009
Promethazine	Pulmonary valve stenosis	17	413	127	4,982	1.6 (1.0-2.5)	1.2 (0.7-2.1)	Gilboa et al., 2009
Promethazine	Hypoplastic left heart	5	229	127	4,982	0.9 (0.3-2.1)	0.7 (0.3-1.7)	Gilboa et al., 2009
Promethazine	Coarctation of aorta	6	218	127	4,982	1.1 (0.5-2.5)	1.1 (0.4-2.8)	Gilboa et al., 2009
Promethazine	Cleft lip ± cleft palate	26	1,150	127	4,982	0.9 (0.6-1.4)	0.8 (0.5-1.3)	Gilboa et al., 2009

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Promethazine	Cleft lip ± cleft palate	78	1,374	1,825	38,151	1.2 (0.9-1.8)	1.5 (1.1-2.0)	Bártfai et al., 2008
Promethazine	Cleft palate	8	575	127	4,982	0.5 (0.3-1.1)	0.5 (0.2-1.1)	Gilboa et al., 2009
Promethazine	Cleft palate	34	582	1,825	38,151	1.2 (0.9-1.8)	1.1 (0.7-1.7)	Bártfai et al., 2008
Promethazine	Esophageal atresia	16	217	1,825	38,151	1.6 (1.0-2.6)	1.6 (0.8-3.4)	Bártfai et al., 2008
Promethazine	Intestinal atresia	5	153	1,825	38,151	0.7 (0.3-1.6)	0.8 (0.3-2.4)	Bártfai et al., 2008
Promethazine	Anorectal malformation	3	223	127	4,982	0.5 (0.2-1.7)	0.3 (0.1-1.3)	Gilboa et al., 2009
Promethazine	Anorectal malformation	10	220	1,825	38,151	0.9 (0.5-1.8)	1.8 (0.7-4.7)	Bártfai et al., 2008
Promethazine	Diaphragmatic hernia	10	298	127	4,982	1.3 (0.7-2.6)	1.2 (0.6-2.5)	Gilboa et al., 2009
Promethazine	Gastroschisis	16	473	127	4,982	1.3 (0.8-2.3)	1.7 (0.9-3.1)	Gilboa et al., 2009
Promethazine	Hypospadias	13	864	NR	NR	NR	0.6 (0.3-1.2)	Gilboa et al., 2009
Promethazine	Limb reduction	7	415	127	4,982	0.7 (0.3-1.4)	0.9 (0.4-2.0)	Gilboa et al., 2009
Promethazine	Limb reduction	36	548	1,825	38,151	1.4 (1.0-2.0)	1.5 (0.9-2.4)	Bártfai et al., 2008
Promethazine	Craniosynostosis	15	477	127	4,982	1.2 (0.7-2.1)	1.2 (0.7-2.0)	Gilboa et al., 2009
Salbutamol	Cleft lip only	18	588	101	6,308	1.9 (1.2-3.2)	1.8 (1.1-3.0)	Munsie et al., 2011
Salbutamol	Cleft lip + cleft palate	15	1,129	101	6,308	0.8 (0.5-1.4)	0.8 (0.4-1.3)	Munsie et al., 2011
Salbutamol	Cleft palate	25	912	101	6,308	1.7 (1.1-2.7)	1.7 (1.1-2.6)	Munsie et al., 2011
Salbutamol	Gastroschisis	12	381	85	4,121	1.5 (0.8-2.9)	NR	Lin et al., 2008
Salicylates	Anencephaly	26	107	18	107	1.6 (0.8-3.1)	NR	Richards, 1972
Salicylates	Cleft lip ± cleft palate	46	194	12	189	4.6 (2.3-9.0)	NR	Saxén, 1975
Salicylates	Cleft palate	24	186	13	181	1.9 (0.9-3.9)	NR	Saxén, 1975
Aspirin	Cleft lip ± cleft palate	28	1,374	435	38,151	1.8 (1.2-2.7)	1.1 (0.7-1.6)	Puhó et al., 2007
Aspirin	Cleft lip ± cleft palate	2	656	5,913	575,829	0.3 (0.1-1.2)	NR	Källén, 2003
Aspirin	Cleft palate	10	601	520	38,151	1.2 (0.7-2.3)	1.0 (0.5-2.0)	Puhó et al., 2007
Aspirin	Cleft palate	5	388	5,913	575,829	1.3 (0.5-3.0)	NR	Källén, 2003
Aspirin	Esophageal atresia	1	192	0	192	–	–	Czeizel et al., 2000
Aspirin	Intestinal atresia	3	132	0	132	–	–	Czeizel et al., 2000
Aspirin	Small intestinal atresia	5	127	12	416	1.4 (0.5-4.0)	NR	Werler et al., 2003
Aspirin	Anorectal malformation	4	201	1	201	4.1 (0.4-36.7)	4.3 (0.4-40.9)	Czeizel et al., 2000
Aspirin	Gastroschisis	33	514	143	3,277	1.5 (1.0-2.2)	1.1 (0.7-1.7)	Werler et al., 2009b
Aspirin	Gastroschisis	13	205	12	416	2.3 (1.0-5.1)	NR	Werler et al., 2003
Aspirin	Gastroschisis	7	110	3	220	4.7 (1.2-18.1)	NR	Torfs et al., 1996

Appendix 3.4 (Continued)

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Aspirin	Omphalocele	12	168	235	4,967	1.5 (0.8-2 8)	1 6 (0 9-3.1)	Mac Bird et al, 2009
Aspirin	Hypospadias	29	2,817	35	2,817	0 8 (0 5-1 4)	0.9 (0.5-1 4)	Czeizel et al., 2000
Aspirin	Limb reduction	8	493	2	493	4 0 (0.9-19.2)	4.2 (0.9-19 9)	Czeizel et al., 2000
Aspirin	Transverse limb deficiency	20	373	265	5,958	1 2 (0 8-1 9)	1.2 (0.8-2.0)	Werler et al, 2009a
Salmeterol	Gastroschisis	2	381	11	4,121	2 0 (0.4-8.9)	NR	Lin et al, 2008
Sex hormones	Hypospadias	27	846	12	846	2 3 (1 2-4.4)	NR	Källén et al., 1992
Sex hormones	Limb reduction	15	108	4	108	4 2 (1 3-13 1)	NR	Janerich et al, 1974
Sex hormones	Limb reduction	9	115	4	115	3 5 (0.8-15.3)	NR	Hill et al, 1988
17OHP	Cleft lip ± cleft palate	7	1,374	178	38,151	1 1 (0 5-2.3)	1 1 (0 5-2.3)	Puhó et al, 2007
17OHP	Cleft palate	3	601	166	38,151	1 1 (0 4-3 6)	1 1 (0.4-3.6)	Puhó et al., 2007
17OHP	Anorectal malformation	2	230	NR	230	NR	1 4 (0 2-10 5)	Dudás et al, 2006
17OHP	Hypospadias	17	3,038	NR	3,038	NR	1 2 (0 6-2.3)	Dudás et al, 2006
17OHP	Hypospadias	4	107	0	226	–	–	Sweet et al, 1974
17OHP	Limb reduction	7	548	NR	548	NR	2.2 (0.7-7 0)	Dudás et al, 2006
Allylestrenol	Cleft lip ± cleft palate	91	1,374	2,283	38,151	1.1 (0 9-1.4)	1 1 (0 9-1.4)	Puhó et al, 2007
Allylestrenol	Cleft palate	49	601	2,364	38,151	1.3 (1 0-1 8)	1 4 (1 0-1 8)	Puhó et al, 2007
Fertility treatment	Anencephaly	5	323	15	611	0 6 (0 2-1 7)	0.7 (0.2-2.3)	Whiteman et al, 2000
Fertility treatment	Spina bifida	7	302	15	611	0.9 (0.4-2.1)	0.8 (0 3-2.7)	Whiteman et al., 2000
Fertility treatment	Cleft lip ± cleft palate	2	656	1,503	575,829	1.2 (0 3-4 7)	NR	Källen, 2003
Fertility treatment	Cleft palate	1	388	1,503	575,829	1.0 (0.1-7.0)	NR	Källén, 2003
Fertility treatment	Limb reduction	6	274	0	274	–	–	Czeizel et al, 1983
Clomiphene	Anencephaly	5	323	13	611	0 7 (0 3-2 0)	0.8 (0.3-2 7)	Whiteman et al, 2000
Clomiphene	Anencephaly	9	329	94	6,500	1.9 (1.0-3 8)	2 3 (1 1-4 7)	Reefhuis et al, 2011
Clomiphene	Encephalocele	4	133	94	6,500	2 1 (0 6-5 7)	2.7 (0.9-7.6)	Reefhuis et al, 2011
Clomiphene	Spina bifida	6	302	13	611	0.9 (0.4-2 5)	0.8 (0.2-3 0)	Whiteman et al, 2000
Clomiphene	Spina bifida	9	788	94	6,500	0.9 (0.5-1 8)	0.8 (0.4-1 8)	Reefhuis et al, 2011
Clomiphene	Cataract	5	208	NR	NR	1.6 (0.8-3 9)	1.3 (0.5-3 4)	Reefhuis et al., 2011
Clomiphene	Anotia/microtia	7	383	94	6,500	1.3 (0.6-2.8)	2.1 (1 0-4 7)	Reefhuis et al, 2011
Clomiphene	Transposition of great vessels	3	443	94	6,500	0 5 (0.1-1 5)	0 4 (0 1-1 4)	Reefhuis et al, 2011
Clomiphene	Perimembranous VSD	31	1,333	94	6,500	1 6 (1.1-2.4)	1.5 (1.0-2.3)	Reefhuis et al, 2011
Clomiphene	Muscular VSD	5	170	NR	NR	4.2 (0.9-18.3)	4 9 (1 4-16 8)	Reefhuis et al, 2011

Drug	Birth defect	Cases		Controls		Crude OR	Adjusted OR	Reference
		Exposed	Total	Exposed	Total	(95% CI)	(95% CI)	
Clomiphene	Atrial septal defect	39	1,934	94	6,500	1.4 (1.0-2.0)	1 5 (1 0-2 3)	Reefhuis *et al.*, 2011
Clomiphene	Atrioventricular septal defect	3	170	94	6,500	1.2 (0 3-3 8)	1 1 (0.3-3.5)	Reefhuis *et al*, 2011
Clomiphene	Tetralogy of Fallot	11	630	94	6,500	1.2 (0.6-2.3)	1 1 (0 6-2 1)	Reefhuis *et al.*, 2011
Clomiphene	Pulmonary valve stenosis	16	829	NR	NR	1 3 (0.7-2 2)	1.3 (0 7-2.2)	Reefhuis *et al*, 2011
Clomiphene	Aortic stenosis	9	243	94	6,500	2.6 (1 2-5 3)	1 9 (0.9-4.0)	Reefhuis *et al*, 2011
Clomiphene	Hypoplastic left heart	8	343	94	6,500	1 6 (0.8-3 4)	1.3 (0 6-2.8)	Reefhuis *et al*, 2011
Clomiphene	Coarctation of aorta	4	120	20	3,572	6.1 (2.1-18 2)	4 5 (1.5-14.0)	Wollins *et al*, 2011
Clomiphene	Coarctation of aorta	20	603	94	6,500	2 3 (1.4-3.8)	1.8 (1 1-3 0)	Reefhuis *et al*, 2011
Clomiphene	Cleft lip ± cleft palate	26	1,698	NR	NR	1.0 (0.7-1 6)	1.1 (0 7-1.8)	Reefhuis *et al*, 2011
Clomiphene	Cleft lip ± cleft palate	5	1,374	NR	1,374	1.5 (0.4-5.4)	2 3 (0 6-8 9)	Bánhidy *et al*, 2008
Clomiphene	Cleft palate	10	883	NR	NR	0 8 (0.4-1.5)	0.8 (0 4-1 5)	Reefhuis *et al.*, 2011
Clomiphene	Esophageal atresia	16	389	94	6,500	2.9 (1.7-5 0)	2.3 (1.3-4 0)	Reefhuis *et al.*, 2011
Clomiphene	Small intestinal atresia	4	257	94	6,500	1.1 (0.3-2.9)	1.4 (0 5-3 8)	Reefhuis *et al*, 2011
Clomiphene	Anorectal malformation	10	592	94	6,500	1.2 (0.6-2 3)	1.2 (0 6-2 3)	Reefhuis *et al.*, 2011
Clomiphene	Biliary atresia	3	105	94	6,500	2 0 (0.4-6 2)	1.4 (0.3-5.8)	Reefhuis *et al.*, 2011
Clomiphene	Diaphragmatic hernia	9	488	94	6,500	1 3 (0.6-2.6)	1.2 (0 6-2.4)	Reefhuis *et al*, 2011
Clomiphene	Omphalocele	9	254	94	6,500	2 5 (1 1-5.0)	2 2 (1.1-4 5)	Reefhuis *et al.*, 2011
Clomiphene	Hypospadias	36	1,177	NR	NR	2 3 (1 4-3 5)	1.5 (0 9-2.3)	Reefhuis *et al*, 2011
Clomiphene	Hypospadias	3	319	59	3,190	0 5	0.5 (0.2-1.5)	Sørensen *et al*, 2005
Clomiphene	Hypospadias	10	3,038	NR	3,038	1 4 (0 6-3 1)	1.7 (0 7-4 2)	Bánhidy *et al*, 2008
Clomiphene	Transverse limb deficiency	10	402	94	6,500	1.7 (0 9-3.4)	1.8 (0.9-3.6)	Reefhuis *et al*, 2011
Clomiphene	Craniosynostosis	30	764	94	6,500	2 8 (1 8-4 2)	1 9 (1 2-3 0)	Reefhuis *et al*, 2011
hCG	Cleft lip ± cleft palate	7	1,374	97	38,151	2.7 (0 8-9.4)	2.7 (0.8-9 9)	Dudás *et al*, 2011
hCG	Hypospadias	12	3,038	NR	NR	1.5 (0 7-3 3)	1 7 (0 8-3 8)	Dudás *et al.*, 2011
hCG	Limb reduction	5	548	97	38,151	3.7 (0.7-19 7)	5.3 (0 9-28.3)	Dudás *et al*, 2011
Progestin	Hypospadias	42	500	31	1,284	3 7 (2 3-6 0)	NR	Carmichael *et al*, 2005
Horm pregn. test	Anencephaly	13	122	20	248	1.4 (0.7-2 8)	NR	Laurence *et al*, 1971
Horm pregn. test	Spina bifida	9	149	16	251	0.9 (0.4-2.2)	NR	Laurence *et al*, 1971
Horm pregn. test	Spina bifida	19	100	4	100	5.6 (1.8-17.2)	NR	Gal, 1972
OCs	Anencephaly	7	198	179	4,000	0 8 (0 4-1 7)	0 8 (0 4-1 6)	Waller *et al*, 2010
OCs	Spina bifida	31	434	179	4,000	1 6 (1.1-2.4)	1.4 (0.9-2 1)	Waller *et al*, 2010

Appendix 3.4 (Continued)

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
OCs	Anotia/microtia	11	244	179	4,000	1.0 (0 5-1 9)	0.8 (0 4-1 6)	Waller *et al*, 2010
OCs	Transposition of great vessels	12	230	179	4,000	1 2 (0.6-2 1)	1.3 (0 7-2 3)	Waller *et al*, 2010
OCs	Perimembranous VSD	26	532	179	4,000	1.1 (0 7-1 7)	1 1 (0.7-1 7)	Waller *et al.*, 2010
OCs	ASD secundum	24	489	179	4,000	1.1 (0.7-1 7)	1.1 (0.7-1 7)	Waller *et al.*, 2010
OCs	ASD NOS/OS	6	136	179	4,000	1 0 (0 4-2 3)	1 0 (0 4-2 3)	Waller *et al*, 2010
OCs	Tetralogy of Fallot	15	390	179	4,000	0 9 (0.5-1 5)	0 7 (0 4-1 2)	Waller *et al*, 2010
OCs	Pulmonary valve stenosis	12	341	179	4,000	0.8 (0 4-1.4)	0.7 (0 4-1 3)	Waller *et al*, 2010
OCs	Aortic stenosis	7	102	179	4,000	1.6 (0 7-3 4)	1.7 (0.8-3 9)	Waller *et al*, 2010
OCs	Hypoplastic left heart	16	186	179	4,000	2.0 (1 2-3 4)	2.3 (1 3-4.3)	Waller *et al*, 2010
OCs	Coarctation of aorta	7	196	179	4,000	0.8 (0 4-1 7)	0 7 (0 3-1 6)	Waller *et al*, 2010
OCs	Cleft lip ± cleft palate	59	1,069	179	4,000	1.2 (0.9-1 7)	1.1 (0 8-1.5)	Waller *et al*, 2010
OCs	Cleft lip ± cleft palate	4	656	1,872	575,829	1 9 (0 7-5.0)	NR	Källén, 2003
OCs	Cleft palate	27	557	179	4,000	1 1 (0 7-1 6)	1 1 (0 7-1 6)	Waller *et al*, 2010
OCs	Cleft palate	2	388	1,872	575,829	1 6 (0 4-6.4)	NR	Källén, 2003
OCs	Esophageal atresia	15	244	179	4,000	1.4 (0.8-2.4)	1 4 (0 8-2 4)	Waller *et al.*, 2010
OCs	Small intestinal atresia	6	168	179	4,000	0.8 (0 3-1.8)	0.7 (0 3-1 6)	Waller *et al*, 2010
OCs	Anorectal malformation	13	382	179	4,000	0 8 (0 4-1.3)	0 7 (0.4-1 3)	Waller *et al.*, 2010
OCs	Diaphragmatic hernia	17	301	179	4,000	1 3 (0 8-2.1)	1 3 (0 8-2 2)	Waller *et al*, 2010
OCs	Gastroschisis	40	447	179	4,000	2 1 (1 5-3 0)	1 8 (1 3-2 7)	Waller *et al*, 2010
OCs	Omphalocele	4	162	179	4,000	0 5 (0 2-1.5)	0.6 (0.2-1.5)	Waller *et al.*, 2010
OCs	Hypospadias	16	846	11	846	1.5 (0.7-3.1)	NR	Kallén *et al*, 1991
OCs	Hypospadias	4	734	3	734	1 3 (0 3-6 0)	NR	Källén *et al*, 1991
OCs	Hypospadias	24	706	NR	NR	NR	0.7 (0 5-1.1)	Waller *et al*, 2010
OCs	Hypospadias	13	3,038	87	24,799	1 2 (0 7-2 2)	1 2 (0 7-2 2)	Wogelius *et al*, 2006
OCs	Hypospadias	28	1,186	307	11,010	0 8 (0.6-1 2)	NR	Nørgaard *et al.*, 2009
OCs	Limb reduction	26	460	179	4,000	1 3 (0 8-2 0)	1 1 (0 7-1 7)	Waller *et al*, 2010
OCs	Limb reduction	18	155	1	274	30 2 (5.3-520)	16.6 (4.3-64)	Kricker *et al*, 1986
OCs	Limb reduction	6	108	1	108	6.3 (0.7-53.2)	NR	Janerich *et al.*, 1974
OCs	Limb reduction	6	115	2	115	3 1 (0.6-15 7)	NR	Hill *et al*, 1988
OCs	Isolated limb reduction	29	537	18	537	1 6 (0 9-3 0)	NR	Czeizel and Kodaj, 1995
OCs	Craniosynostosis	19	412	179	4,000	1 0 (0 6-1.7)	1 0 (0 6-1 6)	Waller *et al*, 2010

Drug		Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
			Exposed	Total	Exposed	Total			
	Estrogen	Hypospadias	0	107	4	226	–	–	Sweet *et al*, 1974
	Estrogen	Limb reduction	8	274	2	274	4.1 (0.9-19.4)	NR	Czeizel *et al*, 1983
SSRIs		Anencephaly	9	214	83	4,092	2.0 (1 0-4.3)	2.4 (1.1-5.1)	Alwan *et al*, 2007
SSRIs		Spina bifida	7	457	83	4,092	0 7 (0 3-1 6)	0.7 (0 3-1 7)	Alwan *et al*, 2007
SSRIs		Anotia/microtia	1	253	83	4,092	0.2 (0 0-1 4)	NR	Alwan *et al.*, 2007
SSRIs		Transposition of great vessels	9	309	83	4,092	1.4 (0 7-3.0)	1.4 (0.7-3.0)	Alwan *et al.*, 2007
SSRIs		Perimembranous VSD	18	797	83	4,092	1.1 (0 6-1.9)	1.2 (0 6-1 9)	Alwan *et al*, 2007
SSRIs		ASD secundum type	17	768	83	4,092	1.1 (0 6-1.9)	1 1 (0.6-1 8)	Alwan *et al*, 2007
SSRIs		ASD NOS	5	252	83	4,092	1 0 (0 3-2.4)	1.0 (0.4-2 5)	Alwan *et al*, 2007
SSRIs		Tetralogy of Fallot	10	428	83	4,092	1.2 (0.6-2 3)	1.2 (0.6-2.5)	Alwan *et al*, 2007
SSRIs		Pulmonary valve stenosis	12	480	83	4,092	1 3 (0 6-2.3)	1.3 (0.7-2 4)	Alwan *et al*, 2007
SSRIs		Hypoplastic left heart	3	218	83	4,092	0 6 (0 2-2 2)	0.6 (0.2-2 1)	Alwan *et al*, 2007
SSRIs		Coarctation of aorta	7	358	83	4,092	1.0 (0 3-2.1)	0 8 (0.3-2 0)	Alwan *et al*, 2007
SSRIs		Cleft lip ± cleft palate	22	1,127	83	4,092	1.0 (0 6-1.6)	0 8 (0.5-1 4)	Alwan *et al*, 2007
SSRIs		Cleft lip ± cleft palate	22	704	160	5,860	1.1 (0 7-1.8)	1 5 (0 9-2 5)	Louik *et al*, 2007
SSRIs		Cleft palate	11	620	83	4,092	0 9 (0 4-1.7)	0 8 (0.4-1 5)	Alwan *et al*, 2007
SSRIs		Cleft palate	7	377	160	5,860	0 7 (0 3-1.4)	0.9 (0.4-2 0)	Louik *et al.*, 2007
SSRIs		Esophageal atresia	9	300	83	4,092	1.5 (0.7-3.0)	1.3 (0.6-2.7)	Alwan *et al*, 2007
SSRIs		Esophageal atresia	4	189	160	5,860	0.8 (0 3-2.1)	NR	Louik *et al*, 2007
SSRIs		Intestinal atresia	1	262	83	4,092	0.2 (0 0-1.3)	NR	Alwan *et al.*, 2007
SSRIs		Small intestinal atresia	2	129	160	5,860	0 6 (0.1-2.3)	NR	Louik *et al*, 2007
SSRIs		Anorectal malformation	8	418	83	4,092	1.0 (0 4-2.0)	0.7 (0.3-1.8)	Alwan *et al.*, 2007
SSRIs		Anorectal malformation	7	215	160	5,860	1 2 (0.6-2.6)	1 9 (0.8-4 3)	Louik *et al.*, 2007
SSRIs		Diaphragmatic hernia	10	297	83	4,092	1.7 (0.8-3 3)	1 6 (0.8-3.3)	Alwan *et al*, 2007
SSRIs		Diaphragmatic hernia	6	192	160	5,860	1 1 (0 5-2 6)	1 8 (0 7-4 2)	Louik *et al*, 2007
SSRIs		Gastroschisis	11	413	83	4,092	1 3 (0.7-2.5)	1.3 (0 6-2.6)	Alwan *et al*, 2007
SSRIs		Omphalocele	11	181	83	4,092	3.2 (1.6-6.1)	2 8 (1 3-5 7)	Alwan *et al*, 2007
SSRIs		Omphalocele	3	127	160	5,860	0 9 (0 3-2 7)	1 4 (0 4-4 5)	Louik *et al*, 2007
SSRIs		Limb reduction	9	193	160	5,860	1.8 (0 9-3.5)	1.7 (0.9-4 3)	Louik *et al*, 2007
SSRIs		Transverse limb deficiency	8	346	83	4,092	1 1 (0 5-2.4)	1 2 (0 6-2 6)	Alwan *et al.*, 2007
SSRIs		Craniosynostosis	24	432	83	4,092	2 8 (1 7-4 5)	2 5 (1.5-4 0)	Alwan *et al.*, 2007

Appendix 3.4 (Continued)

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
SSRIs	Craniosynostosis	2	115	160	5,860	0 6 (0 2-2.6)	0.8 (0.2-3.5)	Louik et al, 2007
Citalopram	Cleft lip ± cleft palate	4	704	15	5,860	2.2 (0 7-6 7)	3.2 (0 9-11 9)	Louik et al., 2007
Citalopram	Cleft palate	1	377	15	5,860	1 0 (0.1-7 9)	2.3 (0.4-12.6)	Louik et al, 2007
Citalopram	Anorectal malformation	1	215	15	5,860	1.8 (0 2-13 8)	3 0 (0 3-28.2)	Louik et al, 2007
Citalopram	Diaphragmatic hernia	0	192	15	5,860	–	–	Louik et al, 2007
Citalopram	Omphalocele	0	127	15	5,860	–	–	Louik et al, 2007
Citalopram	Limb reduction	1	193	15	5,860	2 0 (0 3-15 4)	4 0 (0 5-33 9)	Louik et al, 2007
Citalopram	Craniosynostosis	0	115	15	5,860	–	–	Louik et al., 2007
Fluoxetine	Anencephaly	0	214	29	4,092	–	–	Alwan et al, 2007
Fluoxetine	Spina bifida	1	457	29	4,092	0 3 (0.0-2 3)	NR	Alwan et al, 2007
Fluoxetine	Cleft lip ± cleft palate	7	1,127	29	4,092	0 9 (0 4-2 0)	0.9 (0 4-2.1)	Alwan et al, 2007
Fluoxetine	Cleft lip ± cleft palate	11	704	61	5,860	1 5 (0 8-2 9)	1 8 (0 8-3 8)	Louik et al, 2007
Fluoxetine	Cleft palate	5	620	29	4,092	1 1 (0 4-3 0)	1.1 (0 4-3 0)	Alwan et al, 2007
Fluoxetine	Cleft palate	3	377	61	5,860	0.8 (0 2-2 4)	1 0 (0 3-3 5)	Louik et al, 2007
Fluoxetine	Esophageal atresia	5	300	29	4,092	2 4 (0 9-6 2)	2.4 (0 9-6.4)	Alwan et al., 2007
Fluoxetine	Anorectal malformation	1	418	29	4,092	0 3 (0 0-2.5)	NR	Alwan et al., 2007
Fluoxetine	Anorectal malformation	2	215	61	5,860	0.9 (0.2-3.7)	1 4 (0 3-6.1)	Louik et al, 2007
Fluoxetine	Diaphragmatic hernia	2	297	29	4,092	0 9 (0.2-4.0)	NR	Alwan et al, 2007
Fluoxetine	Diaphragmatic hernia	3	192	61	5,860	1.5 (0 5-4.9)	2.0 (0 6-6.9)	Louik et al, 2007
Fluoxetine	Gastroschisis	3	413	29	4,092	1.0 (0.3-3.4)	0.9 (0.3-3 3)	Alwan et al, 2007
Fluoxetine	Omphalocele	3	181	29	4,092	2 4 (0 7-7 8)	1.7 (0 4-7.3)	Alwan et al., 2007
Fluoxetine	Omphalocele	0	127	61	5,860	–	–	Louik et al., 2007
Fluoxetine	Limb reduction	3	193	61	5,860	1 5 (0 5-4 8)	1.7 (0 5-5.7)	Louik et al, 2007
Fluoxetine	Transverse limb reduction	3	346	29	4,092	1.2 (0.4-4.0)	1.3 (0.4-4.4)	Alwan et al, 2007
Fluoxetine	Craniosynostosis	10	432	29	4,092	3 3 (1 6-6 9)	2 8 (1 3-6 1)	Alwan et al, 2007
Fluoxetine	Craniosynostosis	0	115	61	5,860	–	–	Louik et al., 2007
Paroxetine	Anencephaly	5	214	18	4,092	5 4 (2 0-14 7)	5 1 (1 7-15 3)	Alwan et al, 2007
Paroxetine	Spina bifida	0	457	18	4,092	–	–	Alwan et al, 2007
Paroxetine	Cleft lip ± cleft palate	7	1,127	18	4,092	1.4 (0.6-3.4)	1 3 (0 5-1.3)	Alwan et al, 2007
Paroxetine	Cleft lip ± cleft palate	4	704	30	5,860	1.1 (0 4-3 2)	1 2 (0 4-3 6)	Louik et al, 2007
Paroxetine	Cleft palate	5	620	18	4,092	1.8 (0.7-5 0)	1.7 (0 6-4.8)	Alwan et al., 2007

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Paroxetine	Cleft palate	3	377	30	5,860	1.6 (0 5-5.1)	1.5 (0.4-5.3)	Louik et al, 2007
Paroxetine	Esophageal atresia	1	300	18	4,092	0.8 (0.1-5.7)	NR	Alwan et al., 2007
Paroxetine	Anorectal malformation	2	418	18	4,092	1.1 (0.3-4.7)	NR	Alwan et al , 2007
Paroxetine	Anorectal malformation	1	215	30	5,860	0.9 (0 1-6 7)	1 0 (0.1-7.8)	Louik et al , 2007
Paroxetine	Diaphragmatic hernia	2	297	18	4,092	1.5 (0.4-6.6)	NR	Alwan et al , 2007
Paroxetine	Diaphragmatic hernia	1	192	30	5,860	1 0 (0.1-7 5)	1.2 (0 2-8 9)	Louik et al , 2007
Paroxetine	Gastroschisis	5	413	18	4,092	2.8 (1.0-7 5)	2 9 (1 0-8.4)	Alwan et al , 2007
Paroxetine	Omphalocele	6	181	18	4,092	7 8 (3.0-19 8)	8.1 (3 1-20.8)	Alwan et al , 2007
Paroxetine	Omphalocele	0	127	30	5,860	–	–	Louik et al., 2007
Paroxetine	Limb reduction	1	193	30	5,860	1 0 (0 1-7 5)	1 0 (0 1-8.3)	Louik et al , 2007
Paroxetine	Transverse limb deficiency	2	346	18	4,092	1 3 (0.3-5 7)	NR	Alwan et al , 2007
Paroxetine	Craniosynostosis	5	432	18	4,092	2 7 (1.0-7 2)	2.3 (0 8-6.4)	Alwan et al , 2007
Paroxetine	Craniosynostosis	1	115	30	5,860	1 7 (0.2-12 6)	1.7 (0.2-14.4)	Louik et al., 2007
Sertraline	Anencephaly	4	214	32	4,092	2 4 (0 8-6 9)	3.2 (1 1-9.3)	Alwan et al , 2007
Sertraline	Spina bifida	5	457	32	4,092	1 4 (0 5-3.6)	1.2 (0.4-3 5)	Alwan et al , 2007
Sertraline	Cleft lip ± cleft palate	9	1,127	32	4,092	1 0 (0 5-2 1)	0.9 (0 4-2.0)	Alwan et al , 2007
Sertraline	Cleft lip ± cleft palate	3	704	46	5,860	0 5 (0 2-1.7)	1.1 (0.3-3.8)	Louik et al , 2007
Sertraline	Cleft palate	3	620	32	4,092	0.6 (0.2-2.0)	0.6 (0 2-1 9)	Alwan et al., 2007
Sertraline	Cleft palate	0	377	46	5,860	–	–	Louik et al , 2007
Sertraline	Esophageal atresia	2	300	32	4,092	0.9 (0 2-3.6)	NR	Alwan et al., 2007
Sertraline	Anorectal malformation	4	418	32	4,092	1.2 (0.4-3.5)	0 7 (0.2-2 8)	Alwan et al , 2007
Sertraline	Anorectal malformation	3	215	46	5,860	1.8 (0 6-5.8)	4.4 (1.2-16.4)	Louik et al , 2007
Sertraline	Diaphragmatic hernia	4	297	32	4,092	1 7 (0 6-4 9)	1.8 (0 6-5.3)	Alwan et al , 2007
Sertraline	Diaphragmatic hernia	1	192	46	5,860	0.7 (0.1-4 8)	1 5 (0 2-11 5)	Louik et al , 2007
Sertraline	Gastroschisis	3	413	32	4,092	0 9 (0.3-3 0)	0.9 (0.3-3 3)	Alwan et al , 2007
Sertraline	Omphalocele	3	181	32	4,092	2 1 (0 6-7 0)	1 5 (0.4-6 6)	Alwan et al., 2007
Sertraline	Omphalocele	3	127	46	5,860	3 1 (0 9-10 0)	5 7 (1 6-20.7)	Louik et al , 2007
Sertraline	Limb reduction	3	193	46	5,860	2.0 (0.6-6 5)	3 9 (1 1-13 5)	Louik et al , 2007
Sertraline	Transverse limb deficiency	3	346	32	4,092	1 1 (0 3-3 6)	1 2 (0 4-4 0)	Alwan et al , 2007
Sertraline	Craniosynostosis	6	432	32	4,092	1 8 (0 7-4 3)	1 7 (0 7-4.2)	Alwan et al , 2007
Sertraline	Craniosynostosis	1	115	46	5,860	1 1 (0 2-8 1)	1 8 (0.2-14 9)	Louik et al , 2007

Appendix 3.4 (Continued)

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Sulfasalazine	Cleft lip ± cleft palate	1	1,369	15	38,151	1.9 (0.2-14.1)	NR	Nørgård *et al.*, 2001
Sulfasalazine	Cleft lip ± cleft palate	2	656	512	575,829	3.4 (0.9-13.8)	NR	Källén, 2003
Sulfasalazine	Cleft palate	1	388	512	575,829	2.9 (0.4-20.7)	NR	Källén, 2003
Terbutaline	Cleft lip ± cleft palate	11	1,374	260	38,151	1.2 (0.6-2.2)	1.2 (0.6-2.2)	Puhó *et al.*, 2007
Terbutaline	Cleft palate	12	601	588	38,151	1.3 (0.7-2.3)	1.3 (0.7-2.3)	Puhó *et al.*, 2007
Triptans	Gastroschisis	1	514	8	3,277	0.8 (0.1-6.4)	NR	Werler *et al.*, 2009b
Valproic acid	Hypospadias	6	2,375	11	12,443	2.9 (0.9-8.4)	NR	Rodríguez-Pinilla *et al.*, 2008

17OHP, 17-hydroxyprogesterone; ACE, angiotensin-converting enzyme; ASD, atrial septal defect; AT II, angiotensin II; Ca-channel block., calcium-channel blocker; hCG, human chorionic gonadotropin; horm. pregn. test, hormonal pregnancy test; NR, not reported; NSAID, non-steroidal anti-inflammatory drug; OC, oral contraceptive; SSRI, selective serotonin-reuptake inhibitor; TAPVR, total anomalous pulmonary venous return; VSD, ventricular septal defect.

Appendix 3.5 Results of the case-control studies with malformed controls.

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Acetaminophen	Cleft lip ± cleft palate	6	1,374	112	20,868	0.8 (0.4-1.9)	1.6 (0.9-2.9)	Puhó *et al.*, 2007
Acetaminophen	Small intestinal atresia	73	127	184	318	1.0 (0.6-1.5)	NR	Werler *et al.*, 2003
Acetaminophen	Anorectal malformation	4	116	NR	NR	2.7 (1.0-7.5)	NR	Reefhuis *et al.*, 1999
Acetaminophen	Gastroschisis	120	205	184	318	1.0 (0.7-1.5)	NR	Werler *et al.*, 2003
Benzodiazepines	Cleft lip ± cleft palate	38	826	434	17,164	1.7 (1.1-2.7)°	NR	Lisi *et al.*, 2010
Diazepam	Cleft lip ± cleft palate	10	440	54	2,475	1.0 (0.5-2.1)	NR	Rosenberg *et al.*, 1983
Diazepam	Cleft lip ± cleft palate	43	1,374	475	20,868	1.4 (1.0-1.9)	1.4 (1.0-1.9)	Puhó *et al.*, 2007
Diazepam	Cleft palate	6	596	14	812	0.6 (0.2-1.5)	0.6 (0.2-1.5)	Czeizel *et al.*, 2003
Diazepam	Cleft palate	3	163	54	2,475	0.8 (0.3-2.7)	NR	Rosenberg *et al.*, 1983
Diazepam	Esophageal atresia	6	214	14	812	1.6 (0.6-4.3)	1.6 (0.6-4.4)	Czeizel *et al.*, 2003
Diazepam	Intestinal atresia	7	151	14	812	2.8 (1.1-7.0)	3.5 (1.3-9.2)	Czeizel *et al.*, 2003
Diazepam	Anorectal malformation	8	220	14	812	2.1 (0.9-5.0)	1.8 (0.7-4.6)	Czeizel *et al.*, 2003
Diazepam	Limb reduction	18	545	14	812	1.9 (1.0-3.9)	2.0 (0.9-4.1)	Czeizel *et al.*, 2003
Oxazepam	Cleft lip ± cleft palate	3	277	5	3,737	8.2 (1.3-42.2)	NR	Cornel *et al.*, 1996
Carbamazepine	Spina bifida	8	2,048	10	11,763	4.6 (1.8-11.7)	4.2 (1.5-11.2)	Jentink *et al.*, 2010a
Carbamazepine	TAPVR	0	132	10	11,763	–	–	Jentink *et al.*, 2010a
Carbamazepine	Cleft lip ± cleft palate	1	3,544	10	11,763	0.3 (0.0-2.6)	0.2 (0.0-1.7)	Jentink *et al.*, 2010a
Carbamazepine	Diaphragmatic hernia	1	755	10	11,763	1.6 (0.2-12.2)	1.0 (0.1-8.5)	Jentink *et al.*, 2010a
Carbamazepine	Hypospadias	6	5,393	NR	NR	–	0.5 (0.2-1.8)	Jentink *et al.*, 2010a
Dextromethorphan	Small intestinal atresia	12	127	23	318	1.3 (0.6-2.8)	NR	Werler *et al.*, 2003
Dextromethorphan	Gastroschisis	17	205	23	318	1.2 (0.6-2.2)	NR	Werler *et al.*, 2003
Ibuprofen	Small intestinal atresia	15	127	48	318	0.8 (0.4-1.4)	NR	Werler *et al.*, 2003
Ibuprofen	Gastroschisis	29	205	48	318	0.9 (0.6-1.5)	NR	Werler *et al.*, 2003
Lamotrigine	Cleft palate	1	1,943	NR	NR	1.1 (0.0-6.4)	0.8 (0.0-4.4)	Dolk *et al.*, 2008
Metronidazole	Cleft lip ± cleft palate	20	1,374	197	20,868	1.5 (1.0-2.5)	1.2 (0.7-1.9)	Puhó *et al.*, 2007
Nitrofurantoin	Cleft lip ± cleft palate	11	1,374	120	20,868	1.4 (0.8-2.6)	1.1 (0.6-2.0)	Puhó *et al.*, 2007
Oxprenolol	Cleft lip ± cleft palate	6	1,374	32	20,868	2.9 (1.2-6.8)	2.8 (1.2-6.6)	Puhó *et al.*, 2007
Paroxetine	Ventricular septal defect	1	183	6	611	0.6 (0.1-4.6)	0.5 (0.1-4.2)	Bakker *et al.*, 2010
Phenobarbital	Cleft lip ± cleft palate	12	1,374	118	20,868	1.5 (0.9-2.8)	1.5 (0.8-2.8)	Puhó *et al.*, 2007
Phenytoin	Cleft lip ± cleft palate	10	1,374	31	20,868	4.9 (2.4-10.1)	4.4 (2.1-9.1)	Puhó *et al.*, 2007
Promethazine	Cleft lip ± cleft palate	60	1,374	43	834	0.8 (0.6-1.3)	1.1 (0.7-1.6)	Bártfai *et al.*, 2008

Appendix 3.5 (Continued)

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Promethazine	Cleft palate	34	582	43	834	1.1 (0 7-1.8)	1 1 (0 7-1.7)	Bártfai *et al.*, 2008
Promethazine	Esophageal atresia	16	217	43	834	1.5 (0.8-2.7)	1.3 (0.7-2.3)	Bártfai *et al.*, 2008
Promethazine	Intestinal atresia	5	153	43	834	0.6 (0 2-1 6)	0.6 (0 2-1.5)	Bártfai *et al.*, 2008
Promethazine	Anorectal malformation	10	220	43	834	0.9 (0.4-1 6)	0 8 (0.4-1 6)	Bártfai *et al.*, 2008
Promethazine	Limb reduction	36	548	43	834	1.3 (0.8-2.0)	1.2 (0.7-1.9)	Bártfai *et al.*, 2008
Salbutamol	Hypospadias	5	294	NR	NR	4.4 (1.7-11.5)	NR	Reefhuis *et al.*, 1999
Salicylic acid	Coarctation of aorta	3	142	NR	NR	6 8 (2.0-22.9)	NR	Reefhuis *et al.*, 1999
Salicylic acid	Syndactyly	3	185	NR	NR	5.2 (1.5-17.4)	NR	Reefhuis *et al*, 1999
Aspirin	Transposition of great vessels	52	210	1,856	6,966	0.9 (0.7-1.2)	0.9 (0.6-1.2)	Werler *et al.*, 1989
Aspirin	Coarctation of aorta	34	123	1,856	6,966	1 1 (0.7-1 6)	1.0 (0.6-1 4)	Werler *et al*, 1989
Aspirin	Cleft lip ± cleft palate	28	1,374	300	20,868	1.4 (1.0-2.1)	1.0 (0.7-1 5)	Puhó *et al.*, 2007
Aspirin	Cleft palate	12	601	272	19,428	1.4 (0.8-2.6)	1.0 (0.6-1.8)	Nørgård *et al.*, 2005
Aspirin	Small intestinal atresia	5	127	13	318	1.0 (0.3-2.8)	NR	Werler *et al.*, 2003
Aspirin	Gastroschisis	13	205	13	318	1.6 (0.7-3 5)	NR	Werler *et al*, 2003
Sex hormones	Anencephaly	9	108	NR	NR	0 6 (0.4-1 2)[b]	NR	Lammer and Cordero, 1986
Sex hormones	Spina bifida	22	181	NR	NR	1.0 (0.7-1.6)[b]	NR	Lammer and Cordero, 1986
Sex hormones	Atrial septal defect	17	325	NR	NR	1.8 (1.1-3 0)	NR	Reefhuis *et al.*, 1999
Sex hormones	Cleft lip ± cleft palate	23	200	NR	NR	1.0 (0.6-1.4)[b]	NR	Lammer and Cordero, 1986
Sex hormones	Cleft palate	17	118	NR	NR	1.3 (0.8-2 0)[b]	NR	Lammer and Cordero, 1986
17OHP	Cleft lip ± cleft palate	7	1,374	120	20,868	0.9 (0.4-1.9)	0.9 (0.4-1.9)	Puhó *et al.*, 2007
Allylestrenol	Cleft lip ± cleft palate	91	1,374	1,365	20,868	1.0 (0.8-1 3)	1.0 (0.8-1 3)	Puhó *et al*, 2007
Clomiphene	Anencephaly	5	121	NR	NR	3.7 (1.5-9.2)	NR	Reefhuis *et al.*, 1999
Clomiphene	Atrial septal defect	11	325	NR	NR	3 1 (1 6-5 9)	NR	Reefhuis *et al*, 1999
Clomiphene	Hypospadias	7	392	64	4,538	1.3 (0.6-2.8)	NR	Meijer *et al.*, 2006
Clomiphene	Limb reduction	5	135	NR	NR	3.3 (1 3-8 1)	NR	Reefhuis *et al.*, 1999
hCG	Atrial septal defect	5	325	NR	NR	2.9 (1.1-7 4)	NR	Reefhuis *et al.*, 1999
hCG	Cleft lip ± cleft palate	5	1,374	50	20,868	1.5 (0.6-3 8)	1.5 (0.6-3.9)	Puhó *et al.*, 2007
OCs	Hypospadias	13	3,038	65	11,881	0.8 (0 4-1 4)	0.8 (0 5-1 5)	Wogelius *et al.*, 2006
Terbutaline	Cleft lip ± cleft palate	11	1,374	167	20,868	1.0 (0.5-1.8)	1.0 (0.5-1 8)	Puhó *et al.*, 2007
Valproic acid	Spina bifida	3	203	NR	NR	9.4 (2.6-33 7)	NR	Reefhuis *et al.*, 1999
Valproic acid	Spina bifida	27	2,046	13	11,725	12.0 (6.2-23.4)	16.3 (8.0-33 4)	Jentink *et al.*, 2010b

Drug	Birth defect	Cases		Controls		Crude OR (95% CI)	Adjusted OR (95% CI)	Reference
		Exposed	Total	Exposed	Total			
Valproic acid	Ventricular septal defect	19	11,711	13	11,725	1 5 (0.7-3 0)	1 8 (0 8-3.9)	Jentink et al., 2010b
Valproic acid	Atrial septal defect	19	8,267	13	11,725	2.1 (1.0-4.2)	3.3 (1.4-7.4)	Jentink et al, 2010b
Valproic acid	Tetralogy of Fallot	3	960	13	11,725	2 8 (0.8-9 9)	2.8 (0.5-10.4)	Jentink et al, 2010b
Valproic acid	Pulmonary valve stenosis	1	311	13	11,725	2 9 (0.4-22 3)	2 9 (0 1-19 5)	Jentink et al, 2010b
Valproic acid	Cleft palate	13	2,244	13	11,725	5 2 (2.4-11 3)	5.2 (2 2-12 3)	Jentink et al, 2010b
Valproic acid	Diaphragmatic hernia	2	754	13	11,725	2 4 (0 5-10 6)	2.4 (0 3-10 7)	Jentink et al, 2010b
Valproic acid	Gastroschisis	1	798	13	11,725	1 1 (0.1-8 7)	1.1 (0 0-7.6)	Jentink et al, 2010b
Valproic acid	Hypospadias	32	5,395	NR	NR	–	6 3 (2 6-15 2)	Jentink et al, 2010b
Valproic acid	Polydactyly	9	3,500	13	11,725	2 3 (1 0-5.4)	2 4 (0.9-6 4)	Jentink et al, 2010b
Valproic acid	Craniosynostosis	4	520	13	11,725	7 0 (2.3-21 5)	7.0 (1 7-22 9)	Jentink et al., 2010b
Vitamin A	Ventricular septal defect	2	542	6	2,609	1 6 (0 3-7 8)	NR	Werler et al, 1990

17OHP, 17-hydroxyprogesterone; ASD, atrial septal defect; CI, confidence interval, hCG, human chorionic gonadotropin, NR, not reported, OC, oral contraceptive, OR, odds ratio; TAPVR, total anomalous pulmonary venous return, VSD, ventricular septal defect.
[a] 99% confidence interval
[b] 90% confidence interval

Chapter 4

Hypertensive disorders and antihypertensive medication during pregnancy and the risk of birth defects: a case-control study

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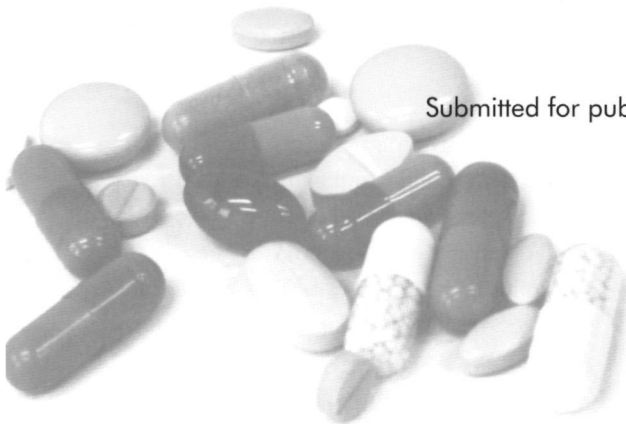
Abstract

Objective To investigate the associations between maternal hypertensive disorders and prenatal exposure to antihypertensive medication and the occurrence of selected birth defects.

Design Case-control study.

Setting Slone Birth Defects Study.

Participants Mothers of 5,349 infants with selected birth defects and 7,253 non-malformed live-born infants as controls.

Main outcome measures Adjusted odds ratios for selected birth defects associated with prenatal exposure to maternal hypertensive disorders or antihypertensive medication. As this was a hypothesis-testing study, we only included specific birth defects that were previously linked to maternal hypertension or antihypertensive medication use in epidemiologic studies.

Results Untreated chronic hypertension was associated with an increased risk of esophageal atresia (adjusted odds ratio 3.2, 95% confidence interval 1.2-8.3), while preeclampsia superimposed on chronic hypertension was more common among cases with septal defects than among control infants. Increased risks were also observed for the use of antihypertensive medication in early pregnancy for chronic hypertension and central nervous system malformations (2.0, 0.9-4.3) and 1st degree hypospadias (2.9, 1.1-7.4). Untreated gestational hypertension was associated with an increased risk of 2nd/3rd degree hypospadias (1.6, 1.0-2.8), whereas untreated preeclampsia was also related to 2nd/3rd degree hypospadias (3.5, 1.8-6.9) and to ventricular septal defects (1.5, 1.1-2.2). Furthermore, treatment for gestational hypertension was associated with ventricular septal defects (2.7, 1.1-6.8) and left-sided cardiovascular defects (4.3, 1.5-12.3).

Conclusions Both treated and untreated specific hypertensive disorders during pregnancy were associated with increased risks of a small number of selected birth defects in the offspring. Further, our findings support the hypothesis that the underlying hypertensive disorder or its subclinical state may increase the risk of malformations, as for several birth defects, manifestation of the hypertensive disorder or its pharmacological treatment took place after the etiologically relevant time period.

Background

Hypertensive disorders in pregnancy include four primary clinical entities: chronic hypertension, gestational hypertension, preeclampsia, and preeclampsia superimposed on chronic hypertension.[1] Collectively, these are common complications during pregnancy with prevalence estimates of 5-10% and they are the leading cause of maternal death in developed countries.[2 3] In addition, all hypertensive disorders of pregnancy are associated with increased risks of neonatal morbidity and mortality.[2]

For severe hypertension in pregnancy, antihypertensive drugs are recommended to prevent maternal and fetal complications, but it is still debated whether mild-to-moderate hypertension should be treated with antihypertensive agents.[4] Several studies have reported associations between early prenatal antihypertensive medication exposure and a number of specific birth defects, including increased risks of cardiovascular malformations,[5 8] severe hypospadias,[9] cleft lip with or without cleft palate,[10] gastroschisis,[11] and malformations of the central nervous system.[6] Others did not find associations between exposure to antihypertensive medication during early pregnancy and the overall occurrence of birth defects,[12 15] these studies may have missed associations with specific birth defects due to lumping of the outcome. Cardiovascular malformations and hypospadias have also been associated with initiation of antihypertensive medication use after the etiologically relevant time period for these defects and with untreated maternal hypertension,[5 7 9] but the authors were unable to distinguish between the different types of hypertensive disorders.

Therefore, we sought to test previously-identified associations between both maternal hypertensive disorders and antihypertensive medication use and the occurrence of specific birth defects looking at specific types of hypertensive disorders in different time windows during pregnancy.

Methods

Study design
We used data from the Slone Birth Defects Study, also known as the Pregnancy Health Interview Study, an ongoing multisite case-control study which was initiated by the Slone Epidemiology Center in 1976 to generate and test hypotheses regarding the risks and relative safety of a wide range of environmental exposures in relation to specific birth defects, with a primary focus on medication use. Live-born or stillborn infants with birth defects were identified either through birth defects registries

(Massachusetts and parts of New York State) or from discharge records of participating hospitals in the areas surrounding Boston (MA), Philadelphia (PA), San Diego (CA), and Toronto (Canada). Controls were live-born infants without birth defects randomly selected from state-wide birth records (Massachusetts) or from study hospitals' discharge lists covering the geographic catchment areas where the cases were identified. Within six months after delivery, and after obtaining informed consent, trained research nurses interviewed the mothers of case and control infants by telephone in either English or Spanish. The interview included questions on demographic characteristics, medical and obstetric history, lifestyle factors, and illnesses, and details of medication use in the two months before pregnancy through the end of pregnancy. The interview lasted approximately 45 minutes and the cooperation rates for mothers of case and control infants were 77% and 70%, respectively.

Case classification
As the nature of this study was hypothesis-testing, we included all specific birth defects that were previously linked to maternal hypertension or antihypertensive medication use during pregnancy in epidemiologic studies. The selected birth defects included central nervous system malformations (one category that included anencephaly, craniorachischisis, spina bifida, encephalocele, cranial meningocele, encephalomyelocele, holoprosencephaly, Dandy-Walker malformation, and hydrocephaly), cardiovascular malformations (situs anomalies and looping defects, conotruncal defects, aortic arch anomalies, ventricular septal defects, atrial septum defect secundum type, atrioventricular septal defects, right-sided defects, Ebstein malformation, left-sided defects, and anomalous pulmonary venous return), cleft lip with or without cleft palate, esophageal atresia with or without tracheoesophageal fistula, small intestinal atresia/stenosis, hypospadias, transverse limb deficiency, and gastroschisis.

Exposure assessment
The interviewers asked detailed questions about the diagnosis, type, timing, and treatment of hypertensive disorders prevalent from two months before pregnancy through birth. Women who reported hypertensive disorders were categorized into the four mutually exclusive groups described in the report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy:[1] chronic hypertension (hypertension diagnosed before pregnancy or before the 20th week of gestation), gestational hypertension (hypertension diagnosed for the first time in the 20th week of gestation or later), preeclampsia, and preeclampsia superimposed on chronic hypertension. Among subjects reporting pharmacological treatment for their hypertension, information was collected on the medication name, start and stop

dates, and duration and frequency of use. Women were considered exposed to antihypertensive medication if they reported any use of antiadrenergic agents, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, diuretics, or direct vasodilators at any time from the month before pregnancy to the end of pregnancy. Based on the groups of hypertensive disorders, two exposure windows for antihypertensive drugs were assessed: "early antihypertensive medication use", defined as any use from one month preconception through the fifth lunar month of pregnancy, representing pharmacological treatment for chronic hypertension with or without preeclampsia, and "late initiation of antihypertensive medication use", defined as medication use between lunar month 6 and birth, representing pharmacological treatment for gestational hypertension and preeclampsia. Women who reported being diagnosed with a hypertensive disorder and did not report antihypertensive medication use were classified as having "untreated hypertension". We considered women who did not report hypertensive disorders and antihypertensive medication to be unexposed to both.

Inclusions and exclusions

For the current study, we selected all cases diagnosed with one of the birth defects of interest without chromosomal abnormalities or associated syndromes ($n=5,757$) and control infants ($n=7,514$) born in the period 1998-2010. We excluded subjects from multiple births (345 cases and 215 controls) and subjects with missing data on diagnosis of hypertensive disorders (1 case and 7 controls) or antihypertensive medication use (3 cases and 1 control). We also excluded 7 case and 5 control mothers who had their first prenatal care visit after gestational week 20 and reported a hypertensive disorder since these subjects could not be reliably classified according to the type of hypertension. In addition, 7 case and 5 control mothers who reported being diagnosed with preeclampsia before the 20th week of gestation were excluded. Finally, we excluded 45 case and 28 control mothers who used antihypertensive medication for an indication other than hypertensive disorders. For hypospadias, only male controls were included in the analyses.

Statistical analysis

We used unconditional multivariable logistic regression analyses with a complete case approach to estimate odds ratios and 95% confidence intervals for the associations between the occurrence of selected birth defects and the four types of maternal hypertensive disorders, stratified by treatment status. As potential confounders we considered maternal age at conception (<35 years or ≥35 years), maternal race or ethnicity (non-Hispanic white or other), maternal education (0-12 years or >12 years), pre-existing diabetes (yes or no), body mass index (BMI) before pregnancy (obese: BMI≥30 or non-obese: BMI<30), parity (0 or ≥1 previous live

births), study centre (5 sites), calendar year of conception, use of folic acid supplements in the month before pregnancy or during the first month of pregnancy (yes or no), use of fertility medication or procedures (yes or no), use of other vasoactive drugs (amphetamines, decongestants, bronchodilators, non-steroidal anti-inflammatory drugs, and antimigraine medication), anticonvulsants, or oral contraceptives in the first trimester (yes or no), and first-trimester nausea/vomiting, binge drinking (≥ 4 per sitting), or smoking (yes or no). Covariates for inclusion in the full models were selected based on associations with both the exposure and the outcome in bivariate analyses. Only factors that altered the effect estimate of the exposure of interest by more than 10% were retained in the final models, except for the following factors that were always included when there were at least 5 exposed cases and controls: study centre, age at conception, race/ethnicity, pre-existing diabetes, BMI before pregnancy, and parity. When there were less than 5 exposed cases or controls, crude odds ratios with Fisher exact 95% confidence intervals were calculated using Episheet.[16] In two secondary analyses, we excluded mothers who had pre-existing diabetes mellitus and infants who had a first-degree relative with the birth defect of interest. The statistical analyses were performed using PASW Statistics version 18.0.2 for Windows (SPSS Inc, Chicago, IL).

Results

A total of 5,349 cases and 7,253 controls were included in this study. Among control mothers, 11.5% ($n=833$) reported a diagnosis of a hypertensive disorder: most were diagnosed with gestational hypertension (56.9%), followed by preeclampsia (21.2%), chronic hypertension (19.9%), and preeclampsia superimposed on chronic hypertension (2.0%). Among control mothers overall, 1.5% ($n=112$) were exposed to antihypertensive medication at some point in time between one month before pregnancy and delivery; for women with a hypertensive disorder, the corresponding proportion was 13.4%.

Table 4.1 shows the maternal and pregnancy characteristics of control mothers who reported a hypertensive disorder. Compared with women without hypertensive disorders, women with chronic hypertension were more likely to be ≥ 35 years at conception, to be non-Hispanic black, and to have a lower level of education, whereas women with gestational hypertension or preeclampsia were more likely to be non-Hispanic white and to be primiparous. Women with chronic hypertension and preeclampsia were more likely to have pre-existing diabetes compared with women without hypertensive disorders. Obesity was more common among women with any of the four types of hypertensive disorders than among women without hypertensive

Table 4.1 Selected maternal and pregnancy characteristics of infants with no major birth defects participating in the Slone Birth Defects Study 1998-2010 by type of hypertensive disorder. Figures are numbers (percentages) of subjects.[a]

Characteristic	No hypertensive disorder (n=6,420)		Chronic hypertension (n=166)		Preeclampsia superimposed on chronic hypertension (n=17)		Gestational hypertension (n=474)		Preeclampsia (n=176)	
Maternal age at conception										
<20 years	468	(7 3)	11	(6.6)	0	(0 0)	30	(6.3)	14	(8.0)
20-34 years	4,771	(74.3)	109	(65.7)	12	(70 6)	355	(74 9)	139	(79.0)
≥35 years	1,162	(18.1)	45	(27.1)	5	(29.4)	89	(18.8)	23	(13 1)
Maternal race or ethnicity										
Non-Hispanic white	4,468	(69 6)	101	(60.8)	11	(64 7)	355	(74 9)	139	(79.0)
Non-Hispanic black	472	(7.4)	28	(16.9)	2	(11.8)	41	(8 6)	15	(8.5)
Hispanic	951	(14 8)	25	(15 1)	4	(23 5)	60	(12.7)	15	(8 5)
Other	520	(8 1)	11	(6.6)	0	(0.0)	17	(3.6)	6	(3.4)
Maternal education										
≤12 years	1,752	(27 3)	66	(39 8)	6	(35.3)	129	(27 2)	45	(25 6)
>12 years	4,662	(72.6)	100	(60.2)	11	(64.7)	345	(72.8)	131	(74 4)
Parity										
0 prior live births	2,646	(41.2)	62	(37.3)	9	(52.9)	253	(53.4)	114	(64 8)
≥1 prior live birth	3,773	(58 8)	104	(62 7)	8	(47 1)	221	(46 6)	62	(35.2)
Pre-existing diabetes										
Yes	34	(0 5)	3	(1 8)	1	(5.9)	2	(0.4)	6	(3 4)
No	6,486	(99 5)	163	(98 2)	16	(94.1)	472	(99 6)	170	(96 6)
BMI before pregnancy										
Underweight (<18.5 kg/m^2)	291	(4 5)	4	(2 4)	0	(0.0)	11	(2 3)	3	(1 7)
Normal weight (18.5-24.9 kg/m^2)	4,009	(62 4)	55	(33 1)	5	(29 4)	243	(51 3)	102	(58 0)
Overweight (25.0-29.9 kg/m^2)	1,286	(20 0)	44	(26 5)	2	(11 8)	117	(24.7)	39	(22.2)
Obese (≥30.0 kg/m^2)	696	(10.8)	57	(34.3)	9	(52.9)	95	(20.0)	27	(15 3)
Use of fertility treatment										
Yes	332	(5.2)	9	(5 4)	2	(11 8)	33	(7 0)	16	(9.1)
No	6,049	(94 2)	155	(93 4)	15	(88 2)	440	(92.8)	160	(90 9)
Periconceptional folic acid use[b]										
Yes	3,102	(48.4)	55	(33.1)	10	(58 8)	242	(51 1)	98	(55 7)
No	3,265	(50 7)	110	(66 3)	7	(41 2)	229	(48 3)	78	(44 3)

[a] Values for each covariate may not add up due to missing values and rounding
[b] Any time from one month before pregnancy through month 1 of pregnancy.

disorders. Women with preeclampsia were more likely to have used fertility treatments and women with chronic hypertension were less likely to have used folic acid in the periconceptional period compared with women who reported no hypertensive disorders.

Analyses by the four different types of hypertensive disorders were divided into those with manifestations before pregnancy or early in pregnancy (chronic hypertension and preeclampsia superimposed on chronic hypertension; Table 4.2) and those manifesting later in pregnancy (gestational hypertension and preeclampsia; Table 4.3). Among women with untreated chronic hypertension, we found an increased risk of esophageal atresia (adjusted odds ratio 3.2, 95% confidence interval 1.2-8.3), which we did not find among women with preeclampsia superimposed on chronic hypertension or among users of early antihypertensive medication for chronic hypertension (Table 4.2). However, the latter may increase the risks of central nervous system malformations (2.0, 0.9-4.3) and 1st degree hypospadias (2.9, 1.1-7.4). For ventricular septal defects, both treated and untreated preeclampsia superimposed on chronic hypertension was associated with an increased risk (3.9, 1.3-11.7 and 3.7, 1.3-10.7, respectively). Although based on small numbers of exposed cases, untreated preeclampsia superimposed on chronic hypertension was also associated with an increased risk of atrial septum defect secundum type (crude odds ratio 7.1, Fisher exact 95% confidence interval 1.5-28.0), and possibly with left-sided cardiovascular malformations (4.9, 0.8-21.4), whereas early antihypertensive medication use for this hypertensive disorder increased the risks of left-sided defects (5.1, 1.1-18.2), cleft lip with or without cleft palate (4.1, 0.9-14.6), and small intestinal atresia (10.3, 1.1-50.5).

Among women with hypertensive disorders that became apparent later in pregnancy (Table 4.3), untreated gestational hypertension was associated with increased risks of esophageal atresia (adjusted odds ratio 1.5, 95% confidence interval 0.9-2.8), small intestinal atresia (1.5, 0.9-2.7), and $2^{nd}/3^{rd}$ degree hypospadias (1.6, 1.0-2.8). The odds ratio for $2^{nd}/3^{rd}$ degree hypospadias was much higher for late initiation of antihypertensive medication treatment for gestational hypertension, but was based on only two exposed cases (crude odds ratio 6.0, Fisher exact 95% confidence interval 0.6-30.3). A decreased risk of gastroschisis (0.3, 0.1-0.6) was found for untreated gestational hypertension. In contrast, treated gestational hypertension was associated with cardiovascular malformations overall (adjusted odds ratio 2.3, 95% confidence interval 1.0-4.9), specifically ventricular septal defects (2.7, 1.1-6.8) and left-sided defects (4.3, 1.5-12.3). Cardiovascular malformations were also associated with untreated preeclampsia, particularly aortic arch malformations (2.3, 0.9-5.6),

Table 4.2 Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for the associations between chronic hypertension and preeclampsia superimposed on chronic hypertension, stratified by treatment group, and selected birth defects, Slone Birth Defects Study, 1998-2010.

Birth defect group	Total No.	Chronic hypertension Untreated No.	Chronic hypertension Untreated OR (95% CI)[a]	Chronic hypertension Antihypertensive medication: early use No.	Chronic hypertension Antihypertensive medication: early use OR (95% CI)[b]	Preeclampsia superimposed on chronic hypertension Untreated No.	Preeclampsia superimposed on chronic hypertension Untreated OR (95% CI)[a]	Preeclampsia superimposed on chronic hypertension Antihypertensive medication: early use No.	Preeclampsia superimposed on chronic hypertension Antihypertensive medication: early use OR (95% CI)[b]
Controls	7,281	99	Reference	63	Reference	7	Reference	9	Reference
Central nervous sytem malformations	413	6	1 0 (0 4-2 4)[c]	9	2.0 (0.9-4 3)	2	5 2 (0 5-27.3)	2	4.0 (0.4-19.5)
Cardiovascular malformations	3,298	52	1.2 (0.9-1 8)	37	0.9 (0 6-1 4)	13	3 7 (1 4-9 7)	14	2 6 (1 0-6 8)
Situs anomalies and looping defects	160	3	1 4 (0 3-4 4)	2	1.5 (0.2-5.7)	0	–	0	–
Conotruncal defects	757	11	1.2 (0 6-2 3)	9	1.3 (0 6-2 6)	2	2 8 (0 3-14 7)	0	–
Aortic arch anomalies	100	2	1 5 (0.2-5.9)	1	–	0	–	0	–
Ventricular septal defects	1,619	24	1 2 (0.7-1 9)	18	0.8 (0 4-1.5)	6	3 9 (1.3-11 7)[d]	9	3 7 (1.3-10 7)
Atrial septal defect secundum type	599	13	1 6 (0 9-3 0)	9	1.5 (0 7-3.2)	4	7 1 (1 5-28 0)	1	–
Atrioventricular septal defect	115	0	–	1	–	0	–	0	–
Right-sided defects	545	7	1 0 (0.5-2 2)[c]	4	0 8 (0 2-2 3)	0	–	0	–
Ebstein malformation	47	0	–	0	–	0	–	0	–
Left-sided defects	658	8	0.9 (0.4-2 0)	8	1 1 (0 5-2.5)[e]	3	4 9 (0.8-21 4)	4	5 1 (1 1-18 2)
Anomalous pulmonary venous return	90	0	–	1	–	0	–	0	–
Cleft lip ± cleft palate	798	7	0 6 (0.3-1.4)[f]	8	1.1 (0.5-2.4)[g]	0	–	4	4 1 (0 9-14.6)
Esophageal atresia	144	5	3 2 (1.2-8 3)	2	1.8 (0 2-6.9)	0	–	0	–
Small intestinal atresia	169	2	0.9 (0.1-3 6)	2	1 5 (0 2-5.7)	0	–	2	10.3 (1 1-50.5)
Hypospadias	481	1	–	8	1 9 (0 8-4.3)[e]	1	–	2	2 4 (0 2-12.4)
1st degree hypospadias	269	1	–	6	2 9 (1 1-7 4)[e]	0	–	1	–
2nd/3rd degree hypospadias	173	0	–	2	1.4 (0 2-5.4)	1	–	1	–
Transverse limb deficiency	73	1	–	0	–	0	–	0	–
Gastroschisis	253	2	0.5 (0.1-2.0)	0	–	0	–	0	–

[a] Adjusted for study centre, age at conception, race/ethnicity, parity, and prepregnancy BMI For exposure groups with <5 exposed cases, presented ORs are crude ORs with Fisher exact 95% CIs.

[b] Adjusted for study centre, age at conception, race/ethnicity, parity, pre-existing diabetes, and prepregnancy BMI For exposure groups with <5 exposed cases, presented ORs are crude ORs with Fisher exact 95% CIs

[c] Adjusted for study centre, race/ethnicity, parity, and prepregnancy BMI only

[d] Adjusted for race/ethnicity, parity, and prepregnancy BMI only

[e] Adjusted for age at conception, race/ethnicity, parity, pre-existing diabetes, and prepregnancy BMI only

[f] Adjusted for race/ethnicity and parity only.

[g] Adjusted for age at conception, race/ethnicity, parity, and prepregnancy BMI only.

Table 4.3 Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for the associations between gestational hypertension and preeclampsia, stratified by treatment group, and selected birth defects, Slone Birth Defects Study, 1998-2010.

Birth defect group	Total No.	Gestational hypertension				Preeclampsia			
		Untreated		Antihypertensive medication: late initiation		Untreated		Antihypertensive medication: late initiation	
		No.	OR (95% CI)[a]	No.	OR (95% CI)[b]	No.	OR (95% CI)[a]	No.	OR (95% CI)[c]
Controls	7,281	456	Reference	14	Reference	159	Reference	16	Reference
Central nervous sytem malformations	413	21	0 8 (0 5-1 2)	1	–	10	1.2 (0.6-2 3)	1	–
Cardiovascular malformations	3,298	183	0.8 (0.7-1.0)	18	2 3 (1 0-4 9)	90	1 3 (1 0-1.7)	8	0 9 (0 4-2.3)
Situs anomalies and looping defects	160	11	1 1 (0.6-2 0)	1	–	5	1 4 (0 5-3 5)[d]	0	–
Conotruncal defects	757	48	1 0 (0.7-1 3)	4	2 8 (0 7-8 9)	18	1.2 (0.7-2.0)	0	–
Aortic arch anomalies	100	5	0.8 (0.3-1.9)[e]	1	–	5	2 3 (0 9-5 6)[f]	0	–
Ventricular septal defects	1,619	93	0.9 (0.7-1 1)	9	2 7 (1 1-6 8)	52	1.5 (1 1-2.2)	5	1 2 (0 4-3 4)
Atrial septal defect secundum type	599	36	0.9 (0.6-1.3)	2	1.8 (0 2-7.8)	10	0 8 (0 4-1 6)	1	–
Atrioventricular septal defect	115	6	0.8 (0 3-1 8)[g]	0	–	3	1.2 (0.2-3 6)	0	–
Right-sided defects	545	35	1.0 (0 7-1 5)	1	–	12	1.2 (0 7-2.2)[h]	2	1 7 (0.2-7 1)
Ebstein malformation	47	0	–	0	–	1	–	0	–
Left-sided defects	658	33	0 7 (0 5-1 1)[h]	5	4.3 (1 5-12 3)[i]	21	1.6 (1 0-2.6)	0	–
Anomalous pulmonary venous return	90	3	0.5 (0 1-1 5)	0	–	1	–	0	–
Cleft lip ± cleft palate	798	51	1.0 (0.7-1 3)	3	2.0 (0 4-7 1)	17	1.1 (0 7-1.9)[h]	1	–
Esophageal atresia	144	15	1 5 (0 9-2.8)[h]	1	–	3	1 1 (0 2-3 3)	1	–
Small intestinal atresia	169	14	1.5 (0 9-2.7)[i]	0	–	6	1.7 (0 7-4 0)[i]	2	5 8 (0.6-25 1)
Hypospadias	481	44	1 5 (1 0-2 1)[h]	3	3 1 (0 5-12 9)	24	2.4 (1 4-4.0)[h]	4	4 1 (0 9-15 4)
1st degree hypospadias	269	21	1.2 (0.8-2.0)[h]	1	–	11	1.8 (0 9-3 7)[h]	1	–
2nd/3rd degree hypospadias	173	18	1.6 (1 0-2.8)[h]	2	6 0 (0 6-30 3)	12	3.5 (1.8-6 9)[h]	2	6 0 (0 6-30 3)
Transverse limb deficiency	73	2	0.4 (0.1-1.6)	1	–	3	1 8 (0 4-5 7)	0	–
Gastroschisis	253	5	0.3 (0 1-0.6)[k]	1	–	6	0 8 (0 3-1 9)[i]	0	–

[a] Adjusted for study centre, age at conception, race/ethnicity, parity, pre-existing diabetes, and prepregnancy BMI. For exposure groups with <5 exposed cases, presented ORs are crude ORs with Fisher exact 95% CIs.

[b] Adjusted for study centre, race/ethnicity, parity, and prepregnancy BMI For exposure groups with <5 exposed cases, presented ORs are crude ORs with Fisher exact 95% CIs.

[c] Adjusted for study centre, age at conception, race/ethnicity, parity, and pre-existing diabetes For exposure groups with <5 exposed cases, presented ORs are crude ORs with Fisher exact 95% CIs.

[d] Adjusted for age at conception, race/ethnicity, parity, pre-existing diabetes, and prepregnancy BMI only

[e] Adjusted for study centre, age at conception, and prepregnancy BMI only

[f] Adjusted for age at conception, race/ethnicity, parity, and prepregnancy BMI only.
[g] Adjusted for study centre, race/ethnicity, parity, pre-existing diabetes, and prepregnancy BMI only.
[h] Adjusted for study centre, age at conception, race/ethnicity, parity, and prepregnancy BMI only.
[i] Adjusted for race/ethnicity, parity, and prepregnancy BMI only.
[j] Adjusted for age at conception, race/ethnicity, and parity only.
[k] Adjusted for study centre, race/ethnicity, and parity only.
[l] Adjusted for study centre, age at conception, parity, and prepregnancy BMI only.

ventricular septal defects (1.5, 1.1-2.2), and left-sided defects (1.6, 1.0-2.6). Furthermore, untreated preeclampsia was associated with increased risks of both 1st degree and 2nd/3rd degree hypospadias (1.8, 0.9-3.7 and 3.5, 1.8-6.9, respectively) with late initiation of antihypertensive medication use for preeclampsia showing higher odd ratios for 2nd/3rd degree hypospadias as was observed for gestational hypertension (based on two exposed cases, crude odds ratio 6.0, Fisher exact 95% confidence interval 0.6-30.3).

Due to small numbers, we could not adjust for pre-existing diabetes in all of the above analyses. Therefore, we controlled for pre-existing diabetes by excluding women with this condition in a secondary analysis. Excluding these women did not substantially alter the results of the primary analyses (data not shown). Furthermore, controlling for family history by excluding those infants who had a first-degree relative with the birth defect under study did not materially change the observed associations either.

Discussion

Comparison with previous studies

Although hypertensive disorders and antihypertensive medication use in early pregnancy may directly affect fetal development through vascular disruption or other teratogenic mechanisms,[17] so far only a few studies have been conducted to identify possible associations between the different types of hypertensive disorders and birth defects. In this case-control study, we found a number of associations between untreated hypertensive disorders that manifest before pregnancy or early in pregnancy and birth defects, including associations between untreated chronic hypertension and esophageal atresia and between untreated preeclampsia superimposed on chronic hypertension and septal defects. The former finding supports an hypothesis from a Hungarian population-based case-control study that showed an increased risk of esophageal atresia among women with chronic hypertension.[18] In that study, however, over 95% of women with chronic hypertension were treated with antihypertensive medication, while in the present study the proportion of treated women was much lower and we only found an increased risk associated with untreated chronic hypertension.

The increased risk of central nervous system malformations we observed after early exposure to antihypertensive medication for chronic hypertension is compatible with the association between central nervous system malformations and angiotensin-converting enzyme inhibitors reported by Cooper *et al.*,[6] but was not found in another epidemiologic study.[5] Consistent with a case-control study by Brouwers *et*

al.,[19] in which most cases were diagnosed with distal hypospadias, we found an increased risk of 1st degree hypospadias in relation to early antihypertensive medication use for chronic hypertension. Although we observed associations between early antihypertensive medication use for preeclampsia superimposed on chronic hypertension and ventricular septal defects and left-sided cardiovascular defects, we could not confirm most of the associations between antihypertensive medication use during pregnancy and cardiovascular defects reported previously, either for cardiovascular defects as a group or for specific heart defects.[5 8] Differences in case classification might explain the discrepancies in results.

To test hypotheses regarding hypertensive disorders that become apparent later in pregnancy, we observed increased risks among women with untreated gestational hypertension and esophageal atresia, small intestinal atresia, and hypospadias, and between untreated preeclampsia and cardiovascular defects, specifically ventricular septal defects and left-sided defects, and both 1st degree and 2nd/3rd degree hypospadias. Associations between gestational hypertension and preeclampsia and the occurrence of hypospadias have been reported previously,[20 22] particularly for severe forms of this defect.[19] Caton *et al.* reported increased risks for a number of cardiovascular defects, including septal defects, and severe hypospadias for women diagnosed with unspecified hypertensive disorders during pregnancy.[7 9] Comparable with our results, an inverse association between gestational hypertension and gastroschisis was recently reported,[23] but the authors used a different definition for this hypertensive disorder. Furthermore, we observed increased odds ratios for having an infant with a ventricular septal defect or left-sided defect among women with late initiation of antihypertensive medication for gestational hypertension. This could reflect confounding by the severity of the hypertensive disorder, which we were unable to control for.

What is common to the birth defects associated with gestational hypertension or preeclampsia is that they all develop prior to the 20th week of gestation. This hampers a causal interpretation as manifestation of these hypertensive disorder and initiation of treatment occurred after the etiologically relevant time period for these defects. Reverse causation might explain the associations between gestational hypertension and preeclampsia and the occurrence of some birth defects, but it is very unlikely that cardiovascular malformations and hypospadias in the unborn child are risk factors for maternal hypertensive disorders. The hypothesis that gestational hypertension, preeclampsia, and some birth defects may share similar risk factors seems more plausible. Although the causes of these hypertensive disorders are largely unknown, obesity, diabetes, and high homocysteine levels are thought to play a role in their etiology, especially in preeclampsia.[24 26] These factors have also been associated

with increased risks of birth defects,[27-29] but as we controlled for obesity and diabetes in our analyses, it is improbable that the increased risks observed were due to these shared risk factors. It is more plausible that a number of risk factors are as yet unknown, and we believe that the most likely explanation was put forward by Caton et al.,[9] namely that fetal development may be affected by a "pre-hypertensive" condition which may be present before the hypertensive disorder itself can be diagnosed. Preeclampsia is thought to be preceded by a relatively hypoxic or ischemic placenta,[30] and placental insufficiency, has been associated with increased risks of hypospadias.[31] However, more knowledge on the underlying pathology of gestational hypertension and preeclampsia is needed to identify those factors potentially affecting fetal development.

Strengths and limitations of the study
The Slone Birth Defects Study's standardized interviews administered within six months after birth collect detailed data on the type of hypertensive disorder and antihypertensive medication use during pregnancy. Information bias and in particular recall bias may constrain the validity of case-control studies, but in the presence of recall bias, one would expect to observe associations with most if not all defects, which was not the case in our analyses. We did not have information on the severity of the hypertensive disorder; although treatment status may serve as a proxy measure, differences in risk between treated and untreated hypertension might also be due to confounding by disease severity.

A major strength of this study is that it is the first in which the analyses were stratified by the type of hypertensive disorder. While aggregation of all forms of hypertension may provide greater statistical power, such an approach lacks biologic or clinical relevance. Furthermore, as we have shown, there are indeed differences in risks of specific birth defects according to the type of maternal hypertension. Separately considering each of the four types of hypertension reduces power, however, and we had insufficient power to consider the risks of specific antihypertensive medications. Thus, despite the relatively large sample size of hypertensive subjects, both treated and untreated, the numbers of cases for some specific birth defects among specific hypertensive disorders were small, resulting in imprecise effect estimates for some associations. We did not adjust for the multiple comparisons made as this was an hypothesis-testing exercise, making it less likely that our results are due to chance. As is always the case in observational studies, we cannot completely rule out the possible role of unmeasured or poorly measured confounders.

Conclusions

In this large case-control study, we confirmed a number of previously reported associations between both treated and untreated specific hypertensive disorders during pregnancy and the risks of selected birth defects. Our results support the hypothesis that physiological changes early in pregnancy that manifest in gestational hypertension and preeclampsia may play a role in the etiology of major birth defects, such as cardiovascular malformations and hypospadias. However, more research is needed to unravel which factors are involved in the pathophysiologic mechanisms that may lead to both maternal hypertensive disorders and birth defects.

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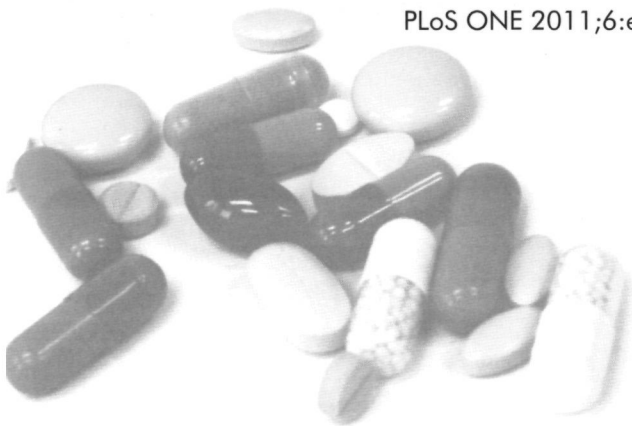
Chapter 5

Exposure to non-steroidal
anti-inflammatory drugs
during pregnancy and the
risk of selected birth defects:
a prospective cohort study

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Nel Roeleveld
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Abstract

Background

Since use of non-steroidal anti-inflammatory drugs (NSAIDs) during pregnancy is common, small increases in the risk of birth defects may have significant implications for public health. Results of human studies on the teratogenic risks of NSAIDs are inconsistent. Therefore, we evaluated the risk of selected birth defects after prenatal exposure to prescribed and over-the-counter NSAIDs.

Methods and findings

We used data on 69,929 women enrolled in the Norwegian Mother and Child Cohort Study between 1999 and 2006. Data on NSAID exposure were available from a self-administered questionnaire completed around gestational week 17. Information on pregnancy outcome was obtained from the Medical Birth Registry of Norway. Only birth defects suspected to be associated with NSAID exposure based upon proposed teratogenic mechanisms and previous studies were included in the multivariable logistic regression analyses. A total of 3,023 women used NSAIDs in gestational weeks 0-12 and 64,074 women did not report NSAID use in early pregnancy. No associations were observed between overall exposure to NSAIDs during pregnancy and the selected birth defects separately or as a group (adjusted odds ratio 0.7, 95% confidence interval 0.4-1.1). Associations between maternal use of specific types of NSAIDs and the selected birth defects were not found either, although an increased risk was seen for septal defects and exposure to multiple NSAIDs based on small numbers (2 exposed cases; crude odds ratio 3.9, 95% confidence interval 0.9-15.7).

Conclusions

Exposure to NSAIDs during the first 12 weeks of gestation does not seem to be associated with an increased risk of the selected birth defects. However, due to the small numbers of NSAID-exposed infants for the individual birth defect categories, increases in the risks of specific birth defects could not be excluded.

Background

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently used for their analgesic, antipyretic, and anti-inflammatory effects. They are among the most common drugs prescribed in the first trimester of pregnancy,[1] and over-the-counter use of NSAIDs is also very widespread during pregnancy with prevalence estimates up to 19%.[2 3] NSAIDs act as an inhibitor of cyclooxygenases (COXs), which catalyze the conversion of arachidonic acid to prostaglandins. Two isoforms of this enzyme have been identified: COX-1 and COX-2. The anti-inflammatory effects of NSAIDs are the result of COX-2 inhibition, while the adverse effects of non-selective NSAIDs are mainly due to the inhibition of COX-1.[4] Results of animal studies suggest that COX-1 inhibition also may lead to cardiac, midline, and diaphragm defects.[5 6]

Since NSAID use during pregnancy is common, even small increases in the risk of birth defects may have significant implications for public health. Results of human studies on the teratogenic risks of first trimester NSAID use are inconsistent. Recent epidemiologic investigations showed an increased risk of congenital heart defects, especially cardiac septal defects, and orofacial clefts,[7 9] while others did not find such effects.[3 10] The aim of this study was to evaluate associations between maternal NSAID use during the first 12 weeks of gestation and the occurrence of selected birth defects using data from the Norwegian Mother and Child Cohort Study (MoBa), which includes information on both prescribed and over-the-counter NSAID use.

Methods

Ethics statement
The study was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All participants gave their written informed consent.

Study population and data collection
MoBa is a prospective cohort study conducted by the Norwegian Institute of Public Health, which enrolled women in early pregnancy between 1999 and 2007. The objective of this study is to estimate the effects of a wide range of exposures during pregnancy on pregnancy outcome and maternal and child health.[11] During the enrolment period, participating hospitals and maternity units weekly provided lists of names and addresses of pregnant women living in Norway who requested routine ultrasound examination. The Norwegian Institute of Public Health subsequently sent these women a postal invitation to participate in MoBa, which included an information brochure, an informed consent form, and the first questionnaire,

together with appointments for routine ultrasound scanning in gestational weeks 13-17. In this questionnaire, questions were asked about sociodemographic characteristics, maternal health, medication use, lifestyle factors, and occupational exposures during the six months prior to pregnancy and during the current pregnancy. The overall participation rate was 43.5% for pregnancies invited in MoBa.[12] To obtain information on pregnancy outcome, data from MoBa were linked to records in the Medical Birth Registry of Norway (MBRN) using the women's personal identification number. MBRN data are obtained by mandatory, standardized forms filled out by midwives, obstetricians, and/or pediatricians, and include detailed medical information regarding the health of both mother and newborn originating from medical records. All births that take place in Norway after gestational week 16 (after week 12 from 2002 onwards), including fetal deaths and elective terminations of pregnancy, are recorded in the MBRN.[13] For the current study, data were available for women enrolled in the period 1999-2006.

Exposure and outcome definitions
Information on the type and timing of both prescribed and over-the-counter NSAID use was available from the questionnaire. If a woman reported use of an NSAID in the six months before or during pregnancy, she could specify five exposure windows: before pregnancy, gestational weeks 0-4, 5-8, 9-12, and 13+ (until completion of the first MoBa questionnaire). We defined NSAID exposure as use of any NSAID (Anatomical Therapeutic Classification code M01A or N02BA)[14] during gestational weeks 0-12. Women were considered non-exposed if they did not report use of any NSAIDs during pregnancy in the first MoBa questionnaire. In addition, the women were asked to report the number of days NSAIDs were taken. However, this question was completed by a minority of women and during the exploratory data analyses the data appeared to be highly unreliable. Therefore, they were not included in this study.

Only birth defects diagnosed by pediatricians and/or geneticists in the first week after birth or while the infants were in the hospital during their first year of life are included in the MBRN records. Birth defects are coded according to the International Classification of Diseases, 10th Revision (ICD-10).[15] For this study, the outcome of interest was the presence of major birth defects that may result from NSAID exposure during pregnancy based upon proposed teratogenic mechanisms and previous epidemiologic studies.[16] These selected birth defects (ICD-10 code) included neural tube defects (Q00, Q01, and Q05), congenital heart defects, subdivided into conotruncal heart defects (Q20.0, Q20.1, Q20.3, Q21.3, Q21.4, and Q25.5-Q25.7 with Q21.0) and septal defects (Q21.0-Q21.2 and Q21.4), orofacial clefts (Q35-Q37), esophageal defects (Q39), anorectal malformations (Q42),

diaphragmatic hernia (Q79.0), abdominal wall defects (Q79.2 and Q79.3), and amniotic bands (Q79.80). For classification of cases into isolated (no other major unrelated birth defect) and multiple birth defects (more than one unrelated major birth defect), the guidelines reported by Rasmussen *et al.*[17] were followed. Infants without any major birth defect were considered unaffected.

Statistical analysis

Our study population consisted of all women who completed the first MoBa questionnaire between 1999 and 2006 for whom data on pregnancy outcome from the MBRN were available (n=69,929). Mothers with pre-existing diabetes were excluded from the analyses because of the known association between this condition and birth defects.[18,19] Case infants with chromosomal abnormalities and mothers with multiple gestations or missing data on the timing of NSAID use (before or during pregnancy) were excluded as well.

Crude results were calculated as odds ratios (ORs) with 95% confidence intervals (CIs). We performed multivariable logistic regression analyses using a complete case analysis approach to estimate the risk of selected birth defects associated with NSAID exposure during the first 12 weeks of gestation, adjusted for maternal age at delivery (in years), maternal education (12 years or less vs. more than 12 years), parity (no previous live births vs. one or more previous live births), presence or absence of a history of miscarriages, stillbirths, or induced abortions, prepregnancy body-mass index (weight in kilograms divided by the square of height in meters; less than 25 vs. 25 or more), any maternal folic acid use from four weeks before pregnancy through week 8 of gestation, and fever and any maternal smoking during gestational weeks 0-12. Adjusted ORs were only calculated if at least three exposed cases were available. We used the same potential confounder set in all models, except when small numbers or a relatively high proportion of missing values ($\geq$10% in either the group of affected or unaffected infants) prevented us from including one or more co-variables. In secondary analyses, we performed crude and adjusted analyses to assess the associations between the different types of NSAIDs (non-selective NSAIDs, acetic acid derivatives, and propionic acid derivatives) and four specific NSAIDs (diclofenac, ibuprofen, naproxen, and aspirin) and the occurrence of the selected birth defects. We also evaluated the effects of exposure to multiple NSAIDs during gestational weeks 0-12 on the risk of the selected birth defects. Additional analyses were performed on time window-specific exposure to NSAIDs. In sensitivity analyses, we assessed whether restricting the analyses to women without pre-existing diseases (asthma, hypertension, or epilepsy) or to infants with isolated defects only changed the effect estimates. Furthermore, we determined whether clustering due to enrolment in MoBa of multiple pregnancies by one woman influenced the results by including

primiparae only, as we did not have information on the number of times a particular woman participated in MoBa. Finally, we estimated the potential effect of bias resulting from the relatively low response rate on the results. All statistical analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL).

Results

Of the 69,929 pregnant women with complete information, 2,038 had one or several of the following exclusion criteria: pre-existing diabetes (n=390), diagnosis of a chromosomal abnormality (n=121), multiple gestation (n=1,290), and missing information on the timing of NSAID use (n=256). Consequently, the final study population consisted of 67,891 women. A total of 6,972 (10.3%) women reported use of an NSAID in the six months before pregnancy and 3,023 (4.5%) women reported NSAID use during the first 12 weeks of gestation. Women who reported NSAID use in gestational weeks 13+ only (n=569) and women who did not report the exact timing of NSAID use during pregnancy (n=225) were omitted from further analyses. Ibuprofen was most commonly used (3.4%), followed by aspirin (0.5%), diclofenac (0.3%), and naproxen (0.2%). Most NSAID-using women used one NSAID (97.2%). Women who used NSAIDs during gestational weeks 0-12 were less likely to have more than 12 years of education, to be married or cohabiting, and to have had a previous live birth compared to non-using women (Table 5.1). NSAID-users were more likely than women who did not use NSAIDs during pregnancy to have had a previous miscarriage, stillbirth, or induced abortion, and to be overweight or obese. However, the absolute differences between the two groups were rather small.

The prevalence of all major birth defects was 2.7% in our cohort (80 affected NSAID-exposed and 1,730 non-exposed infants). The selected birth defects were diagnosed in 638 infants (1.0%). A total of 18 infants had a neural tube defect (including 2 with anencephaly, 2 with encephalocele, and 15 with spina bifida), 435 a congenital heart defect (including 38 with conotruncal defect, 289 with ventricular septal defect, 156 with atrial septal defect, and 7 with atrioventricular septal defect), 134 an orofacial cleft (including 44 with cleft palate and 90 with cleft lip with or without cleft palate), 20 an esophageal defect, 16 an anorectal malformation, 11 a diaphragmatic defect, and 21 an abdominal wall defect (including 6 with omphalocele and 15 with gastroschisis). No infants in our study cohort were diagnosed with amniotic bands. Of the infants with selected birth defects, 42 infants (6.6%) were classified as having multiple defects. Our study cohort included 65,287 infants without a major birth defect.

Table 5.1 Characteristics of women who used and did not use non-steroidal anti-inflammatory drugs (NSAIDs) in gestational weeks 0-12.[a]

Characteristic	NSAID used (n=3,023)		No NSAID used (n=64,074)	
	No.	%	No.	%
Age at delivery				
<20 years	42	1 4	689	1.1
20-29 years	1,320	43.7	28,406	44 3
30-39 years	1,607	53.2	33,774	52 7
≥40 years	54	1 8	1,205	1 9
Education				
<10 years	96	3.2	1,989	3 1
10-12 years	1,134	37.5	21,575	33 7
>12 years	1,639	54 2	37,109	57.9
Other	62	2.1	1,091	1 7
Missing	92	3.0	2,310	3 6
Married/cohabiting				
Yes	2,862	94 7	61,541	96 0
No	143	4.7	2,232	3 5
Missing	18	0 6	301	0 5
Parity				
0 previous live births	1,393	46.1	27,732	43 3
≥1 previous live births	1,629	53.9	36,337	56 7
Missing	1	0.0	5	0 0
Previous miscarriages, stillbirth, or induced abortions				
None	1,874	62 0	41,175	64 3
Miscarriage or stillbirth	572	18 9	11,661	18 2
Induced abortion	401	13 3	7,190	11 2
Induced abortion and miscarriage or stillbirth	123	4 1	2,347	3 7
Missing	53	1 8	1,701	2 7
Prepregnancy body-mass index[b]				
Underweight	74	2 4	1,947	3 0
Normal weight	1,795	59.4	40,534	63 2
Overweight	684	22.6	13,782	21 5
Obese	398	13.2	5,917	9 2
Missing	72	2.4	1,894	3 0
Any folic acid use[c]				
Yes	1,916	63.4	40,611	63 4
No	1,107	36.6	23,463	36 6
Pregnancy outcome				
Live birth, still alive	2,993	99.0	63,622	99 3
Live birth, died during follow-up	10	0 3	158	0.2
Stillbirth	19	0.6	271	0 4
Induced abortion	1	0.0	23	0 0

[a] Data from the Norwegian Mother and Child Cohort Study, 1999-2006. Percentages may not add up to 100% due to rounding

[b] The body-mass index is the weight in kilograms divided by the square of the height in meters underweight <18.5 kg/m², normal weight: 18.5-24.9 kg/m², overweight: 25 0-29.9 kg/m², obese ≥30 kg/m²

[c] Folic acid use is reported from the four weeks prior to pregnancy through week 8 of gestation

Appendix 5.1 shows the characteristics of the 23 infants exposed to NSAIDs in the first 12 weeks of gestation who had any of the selected birth defects. The maternal age at delivery ranged from 25 to 35 years. A total of 21 infants were live born at gestational ages ranging from 34 to 42 weeks, one woman had a miscarriage at 18

weeks of gestation, and one infant was stillborn at a gestational age of 39 weeks. All but five of these infants were exposed to other medications during pregnancy in addition to NSAIDs, of which one infant was exposed to a drug generally considered teratogenic (podophyllotoxin).

The crude and adjusted odds ratios for overall NSAID exposure and the selected birth defects are shown in Table 5.2. Any NSAID use during the first 12 weeks of gestation was not associated with all selected birth defects as a group (adjusted OR 0.7, 95% CI 0.4-1.1) nor with any of the birth defect categories, including any congenital heart defects (adjusted OR 0.9, 95% CI 0.5-1.4), septal defects (adjusted OR 0.8, 95% CI 0.5-1.4), ventricular septal defects (adjusted OR 0.7, 95% CI 0.4-1.4), and atrial septal defects (adjusted OR 1.1, 95% CI 0.5-2.3). For the other groups of birth defects, there were too few exposed cases to reliably estimate adjusted odds ratios. A crude OR of 0.2 (95% CI 0.0-1.1) was seen for orofacial clefts based on one exposed case. Analyses for the three exposure time-windows assessed in the questionnaire separately did not alter these results (Table 5.3), although we saw a slightly increased risk for atrial septal defects after NSAID exposure in gestational weeks 5-8 (adjusted OR 1.6, 95% CI 0.7-3.9).

Table 5.2 Associations between maternal use of non-steroidal anti-inflammatory drugs (NSAIDs) in gestational weeks 0-12 and selected birth defects.[a]

Birth defect	NSAID used (n=3,023)		No NSAID used (n=64,074)		Odds ratio (95% CI)	
	No.	%	No.	%	Crude	Adjusted[b]
No major birth defects	2,943	97 4	62,344	97 3	Reference	Reference
Any selected birth defect	23	0 8	615	1 0	0 8 (0 5-1 2)	0 7 (0 4-1 1)
Neural tube defects	1	0.0	17	0 0	1.2 (0.2-9.4)	–
Congenital heart defects	20	0.7	415	0.6	1.0 (0 7-1.6)	0.9 (0.5-1 4)
Conotruncal heart defects	2	0 1	36	0 1	1 2 (0 3-4 9)	–
Septal defects	18	0.6	394	0 6	1.0 (0.6-1.6)	0.8 (0.5-1 4)
Ventricular septal defect	11	0 4	278	0 4	0 8 (0 5-1.5)	0.7 (0.4-1 4)
Atrial septal defect	8	0.3	148	0 2	1 1 (0 6-2 3)	1.1 (0.5-2 3)[c]
Orofacial clefts	1	0 0	133	0.2	0 2 (0.0-1.1)	–
Esophageal defects	0	0.0	20	0.0	–	–
Anorectal malformations	1	0.0	15	0.0	1 4 (0 2-10 7)	–
Diaphragmatic hernia	0	0.0	11	0.0	–	–
Abdominal wall defects	0	0.0	21	0.0	–	–
Amniotic bands	0	0.0	0	0.0	–	–

[a] Data from the Norwegian Mother and Child Cohort Study, 1999-2006 Infants with multiple selected birth defects were included in all relevant outcome categories. Adjusted analyses were performed if at least three exposed cases were available

[b] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, prepregnancy body-mass index, folic acid use, fever, and smoking.

[c] Adjusted for maternal age at delivery, parity, prepregnancy body-mass index, folic acid use, and smoking

Table 5.3 Associations between maternal use of non-steroidal anti-inflammatory drugs (NSAIDs) during the three exposure windows and selected birth defects.[a]

Birth defect	No NAID used (n=64,074)	NSAID used in gestational weeks 0-4 (n=1,607)		NSAID used in gestational weeks 5-8 (n=1,329)		NSAID used in gestational weeks 9-12 (n=1,422)	
	No.	No.	Adjusted odds ratio (95% CI)[b]	No.	Adjusted odds ratio (95% CI)[c]	No.	Adjusted odds ratio (95% CI)[d]
No major birth defects	62,344	1,561	Reference	1,290	Reference	1,383	Reference
Any selected birth defect	615	10	0.7 (0.4-1.3)	12	0 8 (0.4-1 5)	10	0 7 (0.4-1 3)
Neural tube defects	17	1	–	0	–	1	–
Congenital heart defects	415	9	0.9 (0.5-1.8)[e]	11	1.0 (0.5-2.0)	8	0 9 (0.4-1 8)[f]
Conotruncal heart defects	36	1	–	1	–	2	–
Septal defects	394	8	0 9 (0 4-1.7)[e]	10	0.9 (0.4-1.9)	6	0 7 (0.3-1 6)[f]
Ventricular septal defect	278	6	0.9 (0.4-2 0)[e]	6	1 1 (0 5-2.5)[g]	2	–
Atrial septal defect	148	3	0 8 (0.3-2.7)[h]	5	1.6 (0.7-3.9)[i]	4	1 2 (0.4-3 3)[f]
Orofacial clefts	133	0	–	0	–	1	–
Anorectal malformations	15	0	–	1	–	0	–

[a] Data from the Norwegian Mother and Child Cohort Study, 1999-2006. Infants with multiple selected birth defects were included in all relevant outcome categories. Adjusted analyses were performed if at least three exposed cases were available.

[b] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, prepregnancy body-mass index, folic acid use, and fever

[c] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, prepregnancy body-mass index, folic acid use, fever, and smoking

[d] Adjusted for maternal age at delivery, parity, prepregnancy body-mass index, folic acid use, and fever

[e] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, prepregnancy body-mass index, and folic acid use.

[f] Adjusted for maternal age at delivery, parity, prepregnancy body-mass index, and folic acid use

[g] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, folic acid use, fever, and smoking.

[h] Adjusted for maternal age at delivery, education, parity, prepregnancy body-mass index, and folic acid use.

[i] Adjusted for maternal age at delivery, parity, prepregnancy body-mass index, folic acid use, and smoking

We conducted several secondary analyses to evaluate the effects of exposure to different types of NSAIDs on the occurrence of the selected birth defects (Table 5.4). Restricting the exposed group to infants exposed to non-selective NSAIDs (excluding infants exposed to coxibs only) did not change the results of the primary analyses. No associations were observed between the selected birth defects as a group and exposure to acetic acid derivatives (one exposed case), propionic acid derivatives (adjusted OR 0.7, 95% CI 0.4-1.2), aspirin (adjusted OR 1.1, 95% CI 0.4-3.5), or multiple NSAIDs (crude OR 2.5, 95% CI 0.6-10.1). However, we found an association between exposure to multiple NSAIDs during gestational weeks 0-12 and congenital heart defects (crude OR 3.7, 95% CI 0.9-14.9), in particular septal defects (crude OR 3.9, 95% CI 0.9-15.7), but this observation was based on only two exposed case infants. We did not find associations between any of the other NSAID subgroups and congenital heart defects or septal defects.

The detailed results of the sensitivity analyses are shown in Appendix 5.2. Analyses restricted to women without pre-existing diseases did not alter the results of the primary analyses, nor did restricting the analyses to affected infants with isolated birth defects only. Restricting the primary analyses to primiparae to estimate the effect of clustering due to enrolment in MoBa of multiple pregnancies by one woman did not change the effect estimates substantially, but it did decrease precision. The sensitivity analysis to estimate the potential bias resulting from the relatively low response rate indicated that selective participation of mothers of either exposed, non-exposed, affected, or unaffected infants did not change the effect estimates of the primary analysis. Only in the unlikely event that one of the four exposure-outcome groups was far more likely to participate in MoBa than the other three groups, the NSAID-birth defect associations observed could have been biased due to selection.

Discussion

In this large prospective cohort study, we did not find associations between exposure to any NSAID in the first 12 weeks of gestation and the occurrence of birth defects such as congenital heart defects and orofacial clefts, which were selected based upon results of previous animal and epidemiologic studies and the proposed teratogenic mechanism of NSAIDs. However, we did observe a non-statistically significantly increased risk of septal defects after exposure to multiple NSAIDs in the first 12 weeks of gestation. Furthermore, it should be kept in mind that NSAIDs have been associated with spontaneous abortions[10,20] and that they are contraindicated during the third trimester of pregnancy due to an increased risk of premature closure of the ductus arteriosus.[21,22]

Table 5.4 Secondary analyses of the risk of selected birth defects among infants with exposure to non-steroidal anti-inflammatory drugs (NSAIDs) in gestational weeks 0-12.[a]

NSAID exposure	Total	Any selected birth defect			Congenital heart defect			Septal defects		
		No.	%	Adjusted odds ratio (95% CI)[b]	No.	%	Adjusted odds ratio (95% CI)[b]	No.	%	Adjusted odds ratio (95% CI)[b]
None	64,074	615	1 0	Reference	415	0 6	Reference	394	0.6	Reference
Non-selective NSAIDs	2,964	23	0.8	0 7 (0 4-1 1)	20	0 7	0 9 (0 5-1 5)	18	0 6	0 8 (0.5-1 4)
Acetic acid derivatives	189	1	0 5	–	1	0 5	–	1	0 5	–
Diclofenac	169	1	0.6	–	1	0.6	–	1	0 6	–
Propionic acid derivatives	2,425	19	0 8	0 7 (0.4-1 2)[c]	16	0 7	0 8 (0 5-1.4)	14	0 6	0 7 (0 4-1 3)
Ibuprofen	2,276	19	0.8	0 8 (0 5-1.3)[c]	16	0.7	0.9 (0 5-1.5)	14	0 6	0 7 (0.4-1.4)
Naproxen	166	0	0 0	–	0	0 0	–	0	0 0	–
Aspirin	307	3	1 0	1 1 (0.4-3 5)[d]	3	1.0	1.6 (0.5-5.2)[d]	3	1.0	1.7 (0.6-5 4)[d]
Multiple NSAIDs	87	2	2 3	–	2	2.3	–	2	2 3	–

[a] Data from the Norwegian Mother and Child Cohort Study, 1999-2006. Infants with multiple selected birth defects were included in all relevant outcome categories Adjusted analyses were performed if at least three exposed cases were available.
[b] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, prepregnancy body-mass index, folic acid use, fever, and smoking
[c] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, folic acid use, fever, and smoking.
[d] Adjusted for maternal age at delivery, parity, prepregnancy body-mass index, and folic acid use

Although NSAID use during pregnancy is prevalent, epidemiologic studies on the teratogenic risks are relatively sparse. Recent case-control studies, which may be prone to recall bias, showed possible associations between NSAID exposure and ventricular septal defects,[23] amniotic bands,[24] and gastroschisis.[25] Two cohort studies using data from registries, of which one used reports from the first prenatal care visit and the other lacked information on compliance and over-the-counter use of NSAIDs, generally found no increased risks of birth defects after NSAID exposure,[7,10] but associations with congenital heart defects and orofacial clefts were reported in the former.[7] A third pregnancy register showed increased risks of any birth defect and of septal defects in particular among infants of women who filled a prescription for NSAIDs in the first trimester.[9] In a recent study which used follow-up data of women who contacted Teratology Information Services, an increased risk of major birth defects after exposure to diclofenac in gestational weeks 5-14 could not be excluded (crude OR 2.5; 95% CI 0.9-6.6).[26] To our knowledge, the current study, using data collected in the Norwegian Mother and Child Cohort Study, is the first prospective cohort study to evaluate the teratogenic risk of both prescribed and over-the-counter NSAIDs, thereby avoiding differential misclassification by the outcome of interest often found in retrospective studies.

In secondary analyses, we observed a possible association between exposure to multiple NSAIDs and septal defects with two (2.3%) of the exposed infants being diagnosed with either a ventricular or an atrial septal defect. This finding was based on very small numbers, but there are several reasons why this finding might indicate a truly increased risk. First, all outcomes of interest, including septal defects, were selected based on biologic plausibility. Secondly, mothers of exposed cases used a combination of either ibuprofen and ketoprofen or ibuprofen and diclofenac, which all inhibit COX-1 by more than 60% when COX-2 is inhibited by 80%.[27] Animal studies indicate that especially NSAIDs with a high COX-1/COX-2 ratio may cause birth defects,[5] in particular since COX-1 is expressed in rat embryos during cardiovascular development.[28] Finally, both animal and human studies have indicated an increased risk of septal defects after prenatal NSAID exposure.[9,20,29] However, as cardiac septation takes place between weeks 4 and 7 of development,[30] exposure did not occur during the etiologically relevant period for one of the exposed cases (Appendix 5.1). Therefore, these results should be interpreted with caution and confirmation by other studies is warranted.

The absence of associations between maternal use of NSAIDs during pregnancy and the occurrence of the selected birth defects may partly be due to the relatively low prevalence of exposure in this cohort (4.5%). Studies conducted in the U.S. found much higher prevalence estimates of NSAID use during the first trimester up to

approximately 19%.[2,3] Comparable European data are lacking, but recent reports indicate that NSAID use during pregnancy may be less prevalent in European countries compared to the U.S.,[31,32] especially since NSAIDs are contraindicated in the first and third trimester of pregnancy. In a study using data from the Norwegian Prescription Database, 2.0% of women filled at least one NSAID prescription in the first trimester of pregnancy.[33] Therefore, we believe that the prevalence of NSAID use during the first 12 weeks of gestation found in our study population is accurate. However, the small number of NSAID-exposed infants for the individual birth defect categories remains a limitation, which made lumping of birth defects necessary for power purposes, which, in turn, may have masked true associations between prenatal NSAID exposure and specific birth defects.

The main strength of this study is its longitudinal design which features prospective ascertainment of NSAID use and other covariate information obtained at a median of 17 weeks of gestation. However, as MoBa has a relatively low participation rate (43.5%), selection bias may have occurred. A recent non-response study showed that the prevalence estimates of several exposures and birth outcomes are biased in MoBa, but that estimates of exposure-outcome associations are not biased due to self-selection.[12] Similar results were obtained from a comparable cohort study conducted in Denmark.[34] In addition, the sensitivity analyses showed that selective participation on either exposure or disease status of the infant did not influence our effect estimates. The only scenario in the sensitivity analyses that affected our NSAID-birth defect risk estimates was when one of the four exposure-outcome groups was more likely to participate than the other three groups, but this is highly unlikely in a prospective cohort study. Therefore, we feel that our results on the associations between prenatal NSAID exposure and selected birth defects are not biased by the low participation rate.

Non-differential misclassification of the exposure status may have occurred since data on NSAID use were collected using self-administered questionnaires. In addition, there might be a chance that the lack of an increased risk of the selected birth defects in NSAID-exposed infants is due to the inability to separate occasional users from the more frequent or continuous users. Confounding by indication cannot be excluded completely either, although restricting our analyses to women without pre-existing diseases did not change the results of the primary analyses, in which fever in the first 12 weeks of gestation was also included as a potential confounder.

Several validation studies have been conducted regarding the accuracy of the MBRN, which showed that the ascertainment of congenital malformations varies according to the type of defect and its severity. For the years 2001-2005, 82% of clinically verified

cases of Down syndrome were recorded in the registry.[35] Overall, 71% of cases with severe isolated cleft palate were reported, whereas as little as 11% of cases with mild cleft palate were recorded.[36] The registration of cardiovascular malformations in the MBRN has not been validated nor confirmed by a geneticist or dysmorphologist. Therefore, misclassification of the outcomes of interest may have occurred in the current study, but we do not expect the ascertainment rates to differ between infants who were exposed to NSAIDs in pregnancy and non-exposed infants. However, this may have decreased our study power and may have led to underestimation of our effect estimates, although the total prevalence of major birth defects in our cohort (2.7%) is comparable to the expected prevalence of 3% in most populations.

Conclusions
The findings of this large prospective cohort study showed no associations between exposure to NSAIDs during the first part of pregnancy and the risk of selected birth defects, although an increased odds ratio was seen for septal defects after exposure to multiple NSAIDs. This observation, based on only two exposed cases, was not statistically significant and needs confirmation by other studies. However, due to the small numbers of NSAID-exposed infants for the individual birth defect categories, increases in the risks of specific birth defects could not be excluded.

Acknowledgements

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Appendix 5.1 Characteristics of the 23 infants born with selected birth defects after prenatal exposure to non-steroidal anti-inflammatory drugs (NSAIDs).

Birth defect	Maternal age (years)	Gestat. age (weeks)	Pregnancy outcome	NSAID used	Timing of exposure (gest. wk)	Chronic/serious maternal condition	Other drugs used in first part of pregnancy
Anencephaly, spina bifida	32	18	Miscarriage	Ibuprofen	0-4, 9-12	None	Acetaminophen, ebastine, fluticasone, unspecified allergy/asthma medication
Atrial septal defect	30	39	Live birth	Aspirin	9-12	Endocarditis	Derm. preparation for hemorrhoids
Atrial septal defect	28	37	Live birth	Ibuprofen	5-12	None	Acetaminophen
Atrial septal defect	33	41	Live birth	Ibuprofen	0-4	None	None
Atrial septal defect	31	37	Live birth	Ibuprofen, ketoprofen	5-12	Cardiopathy	Clotrimazole, xylometazoline
Atrial septal defect	35	40	Live birth	Ibuprofen	5-12	None	Acetaminophen
Atrial septal defect, patent ductus arteriosus	34	37	Live birth	Piroxicam	5-8	None	Acetaminophen, phenoxymethylpenicillin
Atrial septal defect, patent ductus arteriosus	27	40	Live birth	Ibuprofen	0-4	None	Cetrizine
Atrial and ventricular septal defects, patent ductus arteriosus	28	39	Live birth	Aspirin	0-8	None	Acetaminophen, alginic acid
Cleft lip	28	40	Live birth	Ibuprofen	9-12	None	None
Imperforate anus	30	42	Live birth	Ibuprofen	5-8	None	Econazole
Tetralogy of Fallot, other anomaly pulmonary artery	31	34	Live birth	Ibuprofen	9-12	None	Cyclizine, oxymetazoline
Transposition of the great vessels, coarctation of aorta	35	39	Stillbirth	Ibuprofen	0-12	None	None
Ventricular septal defect	28	41	Live birth	Ibuprofen	5-8	None	Benzoyl peroxide, budesonide, phenylpropanolamine, podophyllotoxin
Ventricular septal defect	35	40	Live birth	Ibuprofen	5-8	Epilepsy	Lamotrigine, sumatriptan
Ventricular septal defect	27	40	Live birth	Ibuprofen	0-12	None	Acetaminophen
Ventricular septal defect	29	38	Live birth	Aspirin	0-4	Asthma, ventricular tachycardia	Acetaminophen, flecainide, hydrocortisone, unspecified dermatol. preparation

Appendix 5.1 (Continued)

Birth defect	Maternal age (years)	Gestat. age (weeks)	Pregnancy outcome	NSAID used	Timing of exposure (gest. wk)	Chronic/serious maternal condition	Other drugs used in first part of pregnancy
Ventricular septal defect	31	40	Live birth	Ibuprofen	5-8	None	Ferrous sulfate
Ventricular septal defect	32	42	Live birth	Ibuprofen	9 12	None	Acetaminophen, clomitrazole, nitrofurantoin, pivmecillinam
Ventricular septal defect	32	40	Live birth	Diclofenac, ibuprofen	0-4	None	Bumetanide, clotrimazole, lactulose
Ventricular septal defect	25	38	Live birth	Ibuprofen	0-4	Epilepsy	None
Ventricular septal defect	33	41	Live birth	Ibuprofen	5-8	None	None
Ventricular septal defect, patent ductus arteriosus	28	40	Live birth	Ibuprofen	0-4	Asthma, chronic urinary tract infections	Acetaminophen, amitriptyline, clotrimazole, desonide, tramadol

Appendix 5.2 Results of the sensitivity analyses on the effect of prenatal exposure to non-steroidal anti-inflammatory drugs (NSAIDs) and selected birth defects.

A. Excluding women with pre-existing diseases

The main analyses were restricted to women without pre-existing diseases, excluding those women who reported having asthma ($n=4,992$), hypertension ($n=673$), or epilepsy ($n=439$) before the index pregnancy (total $n=6,007$). A total of 61,884 women without pre-existing diseases were included in sensitivity analysis A, of which the results are shown in Table 5A.

B. Restriction to infants with isolated defects only

The main analyses were restricted to case infants with isolated defects only, excluding infants who were diagnosed with multiple defects ($n=42$). A total of 67,055 women were included in sensitivity analysis B. The results are shown in Table 5B.

Table 5A Associations between maternal use of NSAIDs and selected birth defects among women without pre-existing diseases [a]

Birth defect	NSAID used (n=2,716)		No NSAID used (n=58,437)		Odds ratio (95% CI)	
	No.	%	No.	%	Crude	Adjusted[b]
No major birth defects	2,650	97 6	56,872	97 3	Reference	Reference
Any selected birth defect	18	0 7	549	0 9	0.7 (0.4-1.1)	0 6 (0.4-1.0)[c]
Neural tube defects	1	0 0	14	0.0	1.5 (0 2-11 7)	–
Congenital heart defects	16	0 6	372	0 6	0.9 (0 6-1.5)	0.7 (0.4-1.3)
Conotruncal heart defects	2	0 1	32	0 1	1.3 (0 3-5 6)	–
Septal defects	14	0.5	353	0.6	0.9 (0 5-1 5)	0 6 (0 3-1 2)
Ventricular septal defect	7	0 3	254	0 4	0.6 (0 3-1 3)	0.6 (0.3-1 2)[d]
Atrial septal defect	8	0 3	129	0 2	1.3 (0 7-2 7)	1 3 (0 6-2 6)[e]
Orofacial clefts	1	0 0	119	0.2	0.2 (0 0-1 3)	–
Esophageal defects	0	0 0	19	0 0	–	–
Anorectal malformations	0	0 0	15	0 0	–	–
Diaphragmatic hernia	0	0 0	6	0.0	–	–
Abdominal wall defects	0	0.0	19	0.0	–	–

[a] Data from the Norwegian Mother and Child Cohort Study, 1999-2006. Infants with multiple selected birth defects were included in all relevant outcome categories Adjusted analyses were performed if at least three exposed cases were available.
[b] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths or induced abortions, prepregnancy body-mass index, folic acid use, fever, and smoking
[c] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths or induced abortions, folic acid use, fever, and smoking
[d] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths or induced abortions, folic acid use, and fever.
[e] Adjusted for maternal age at delivery, parity, prepregnancy body-mass index, folic acid use, and smoking

Table 5B Associations between maternal use of NSAIDs and selected isolated birth defects.[a]

Birth defect	NSAID used (n=3,023)		No NSAID used (n=64,032)		Odds ratio (95% CI)	
	No.	%	No.	%	Crude	Adjusted[b]
No major birth defects	2,943	97 4	62,344	97 4	Reference	Reference
Any selected birth defect	23	0 8	573	0 9	0.9 (0 6-1 3)	0 7 (0.5-1 2)
Neural tube defects	1	0 0	15	0 0	1 4 (0 2-10 7)	–
Congenital heart defects	20	0.7	387	0.6	1 1 (0 7-1.7)	0 9 (0 6-1 5)
Conotruncal heart defects	2	0 1	30	0 0	1.4 (0 3-5.9)	–
Septal defects	18	0 6	370	0 6	1 0 (0.6-1.7)	0 9 (0.5-1 5)
Ventricular septal defect	11	0 4	266	0 4	0 9 (0 5-1 6)	0 8 (0 4-1.5)
Atrial septal defect	8	0 3	134	0 2	1.3 (0.6-2 6)	1 2 (0 6-2.5)[c]
Orofacial clefts	1	0 0	124	0 2	0 2 (0.0-1.2)	–
Esophageal defects	0	0.0	11	0.0	–	–
Anorectal malformations	1	0.0	10	0.0	2 1 (0.3-16 6)	–
Diaphragmatic hernia	0	0 0	9	0 0	–	–
Abdominal wall defects	0	0.0	19	0.0	–	–

[a] Data from the Norwegian Mother and Child Cohort Study, 1999-2006. Infants with multiple selected birth defects were included in all relevant outcome categories. Adjusted analyses were performed if at least three exposed cases were available.

[b] Adjusted for maternal age at delivery, education, parity, history of miscarriages, stillbirths, or induced abortions, prepregnancy body-mass index, folic acid use, fever, and smoking.

[c] Adjusted for maternal age at delivery, parity, prepregnancy body-mass index, folic acid use, and smoking

C. Restriction to primiparae only

Among women enrolled between 1999-2005, 90.7% participated with one pregnancy, 9.0% with two pregnancies, 0.3% with three pregnancies, and one woman with four pregnancies.[11] However, as we did not have information on which women participated multiple times in MoBa, we included only primiparae in this sensitivity analysis to estimate the potential effect of clustering due to enrolment of multiple pregnancies by one woman. A total of 29,471 primiparae were included in sensitivity analysis C. The results are displayed in Table 5C.

D. Potential effect of bias due to relatively low response rate

Participation rates of less than 100% may introduce selection bias if the expose-disease association is different for participants than for all subjects eligible for inclusion in the study. The participation rate for MoBa was 43.5%, so our target population included 154,246 subjects. Because we did not have prevalence data on NSAID use in the first 12 weeks of gestation and on the selected birth defects among infants eligible for inclusion in our study cohort, we assessed the potential effect of selection bias in a simulation study. The results of this analysis are shown in Table 5D.

Table 5C Associations between maternal use of NSAIDs and selected birth defects among primiparae.[a]

Birth defect	NSAID used (n=1,393) No.	%	No NSAID used (n=27,732) No.	%	Odds ratio (95% CI) Crude	Adjusted[b]
No major birth defects	1,355	97.3	26,884	96 9	Reference	Reference
Any selected birth defect	9	0.6	291	1.0	0.6 (0 3-1.2)	0 6 (0 3-1.2)
Neural tube defects	0	0 0	9	0.0	–	–
Congenital heart defects	7	0 5	200	0.7	0.7 (0 3-1.5)	0.8 (0 4-1 6)
Conotruncal heart defects	0	0 0	20	0.1	–	–
Septal defects	7	0.5	188	0.7	0.7 (0 3-1.6)	0 8 (0 4-1 7)
Ventricular septal defect	5	0.4	134	0.5	0.7 (0 3-1 8)	0 8 (0 3-1.9)
Atrial septal defect	3	0 2	73	0 2	0 8 (0.3-2 6)	0.9 (0.3-2.8)[c]
Orofacial clefts	1	0 1	57	0 2	0 3 (0.0-2.5)	–
Esophageal defects	0	0.0	12	0.0	–	–
Anorectal malformations	1	0 1	8	0.0	2.5 (0 3-19 8)	–
Diaphragmatic hernia	0	0 0	4	0.0	–	–
Abdominal wall defects	0	0.0	14	0 1	–	–

[a] Data from the Norwegian Mother and Child Cohort Study, 1999-2006 Infants with multiple selected birth defects were included in all relevant outcome categories. Adjusted analyses were performed if at least three exposed cases were available.
[b] Adjusted for maternal age at delivery, education, prepregnancy body-mass index, and folic acid use
[c] Adjusted for maternal age at delivery and education.

Table 5D Potential effect of selection bias on the crude effect estimates on the association between prenatal exposure to non-steroidal anti-inflammatory drugs (NSAIDs) and selected birth defects.

	Participation rate (%) among Exp. affected	Non-exp. affected	Exp. unaffected	Non-exp. unaffected	Crude OR (95% CI) in target population (n=154,246) Any selected birth defect	Congenital heart defect	Atrial septal defect
1	43 5	43 5	43 5	43.5	0.8 (0.6-1.0)	1.0 (0.8-1.4)	1 1 (0 7-1 8)
2.	85 0	42 5	85 0	42 5	0.8 (0.5-1.2)	1.0 (0.7-1 6)	1 1 (0 6-2 1)
3.	22.7	45.5	22.7	45 5	0.8 (0.6-1.0)	1 0 (0.8-1.3)	1.1 (0 8-1 6)
4	86 6	86.6	43.3	43.3	0.8 (0.5-1 2)	1.0 (0.7-1 5)	1 1 (0 6-2 2)
5	22 0	22 0	43.9	43.9	0.8 (0 7-1.0)	1.0 (0 8-1 3)	1 1 (0 8-1 6)
6	87 0	43 5	43.5	43.5	0.4 (0.3-0.6)	0.5 (0 3-0 8)	0.6 (0 3-1.1)
7.	43.3	86 6	43 3	43.3	1 6 (1 2-2 1)	2.0 (1 5-2 8)	2.2 (1 4-3 6)
8	42 5	42.5	85.0	42.5	1 6 (1.2-2.1)	2.0 (1 5-2 7)	2.3 (1 5-3 7)
9.	22.8	22 8	22.8	45.9	0 4 (0.3-0 5)	0.5 (0 4-0 6)	0.5 (0 4-0 7)

Scenarios
1. No selection bias.
2 NSAID-using women were twice as likely to participate compared to non-using women
3. Non-using women were twice as likely to participate compared to NSAID-using women.
4. Mothers of affected infants were twice as likely to participate compare to mothers of unaffected infants.
5. Mothers of unaffected infants were twice as likely to participate compared to mothers of affected infants
6 Mothers of exposed affected infants were twice as likely to participate compared to the other groups.
7 Mothers of non-exposed affected infants were twice as likely to participate compared to the other groups
8 Mothers of exposed unaffected infants were twice as likely to participate compared to the other groups
9 Mothers of non-exposed unaffected infants were twice as likely to participate compared to the other groups

Part II

Illicit drug use

Chapter 6

Reproductive health
characteristics of marijuana
and cocaine users: results from
the 2002 National Survey
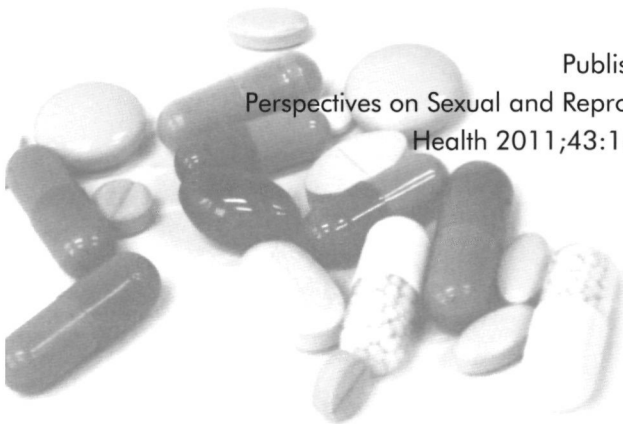of Family Growth

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Nel Roeleveld

Abstract

Context

Illicit drug use is associated with risky sexual behaviors in adolescents and young adults. However, few studies have examined these associations among drug users of all reproductive ages, using a control group of nonusers.

Methods

Associations between marijuana and cocaine use, and outcomes related to sexual behaviors and reproductive health, were assessed using data from the 2002 National Survey of Family Growth. Overall, 4,928 men and 7,643 women aged 15-44 were interviewed. Chi-square tests, t-tests, and multivariable logistic regression analyses were used; in supplementary analyses, men and women were stratified by age-group (25 or younger, and older than 25), to capture the understudied older adults who use drugs.

Results

Twenty-seven percent of men and 16% of women reported use of marijuana or cocaine in the last year. Drug users were younger than nonusers at first vaginal sex (mean, 15.2-16.1 vs. 17.3-17.5 years) and were more likely to have engaged in risky sexual behaviors in the last year, including having had sex with a non-monogamous partner (odds ratios, 3.3-5.2 for men and 2.9-6.5 for women), while high on alcohol or drugs (10.1-18.0 and 8.1-24.2), or in exchange for money or drugs (2.7-2.8 and 2.3-9.2). They also were more likely to have undergone STD testing or treatment. Drug use was associated with risky sexual behaviors in both age-groups.

Conclusion

Programs aimed at reducing sexual risks among drug users should address the behaviors of men and women of all reproductive ages.

Background

In the 2008 National Survey on Drug Use and Health, 14% of Americans aged 12 and older reported illicit drug use in the past year.[1] Use of marijuana was reported most frequently, followed by use of cocaine, hallucinogens, stimulants and heroin. Men reported drug use more often than women (16% vs. 12%). The prevalence of reported use was highest among 18-25 year-olds (34%), but a substantial proportion of Americans aged 26 or older (10%) reported drug use as well. Associations between illicit drug use, risky sexual behaviors, and reproductive health have been studied predominantly in adolescents and young adults (age 25 or younger) or in selected subpopulations. Findings from these studies may apply to older drug users; however, nationally representative data on this population are scarce.

Cross-sectional as well as longitudinal studies have shown associations between illicit drug use among young people and early sexual intercourse,[2 4] multiple sexual partners,[4 6] and inconsistent condom use.[6 8] These risky sexual behaviors put drug-using youth at increased risk for sexually transmitted diseases (STDs; including HIV) and unplanned pregnancies, which may have serious long-term health, social, and economic consequences. For example, having multiple sexual partners - especially when condoms are not used correctly or consistently - increases the risk of contracting STDs; and an untreated STD may lead to pelvic inflammatory disease (PID) and infertility.[9] The direct medical cost of STDs among U.S. 15-24-year-olds was estimated to be $6.5 billion in 2000.[10] Moreover, every new sexual partner increases the risk of acquiring a genital human papillomavirus infection,[11] which may cause genital warts and cervical cancer.[12]

Different models have been proposed to elucidate the associations between drug use and risky sexual behaviors. One model emphasizes that individual personality characteristics leading to risk-taking in general may play a role in the relationship between drug (including alcohol) use among adolescents and young adults.[13,14] Another model explores whether drug-induced impairment of judgment[15] or self-control[16] is associated with risky sexual behaviors; however, studies that considered the timing of drug use in relation to sexual risk-taking do not support this hypothesis.[14,17]

Drug use also could directly affect reproductive health. Cannabinoids, the psychotropic ingredients in marijuana, may decrease testosterone secretion and semen quality and subsequently impair male fertility.[18,19] In animal models, marijuana and its main psychoactive ingredient (commonly abbreviated as THC) disrupt the menstrual cycle and female hormonal secretion. These effects have been inconsistent in humans, however, probably because the timing of marijuana use in

relation to the menstrual cycle has varied [20] Chronic cocaine use has been associated with menstrual cycle abnormalities in rhesus monkeys [21] Although prenatal marijuana use does not seem to be associated with preterm birth or low birth weight,[22 23] cocaine use has been linked to multiple pregnancy complications, including premature rupture of membranes, placental abruption, and low birth weight [24] Prenatal illicit drug exposure also has been associated with some birth defects, such as anencephaly and cleft palate, and long-term developmental problems [25 26]

Substance abuse and responsible sexual behavior have been identified by Healthy People 2010 as leading health indicators for the United States [27] Since drug use may have a profound impact on both sexual behavior and reproductive health, a deeper understanding of their potential associations is needed to strengthen programs focusing on STD prevention The primary aim of this study is to describe the reproductive health characteristics, risky sexual behaviors, and STD experiences associated with illicit drug use among U S men and women of reproductive age (15-44) Supplementary analyses assess these associations separately among younger (aged 15-25) and older (aged 26-44) individuals

Methods

Data and study population
Data for this descriptive study come from the 2002 National Survey of Family Growth (NSFG),[28] a U S population-based survey conducted by the National Center for Health Statistics The NSFG sampling design has been described in detail elsewhere [29] In brief, a multistage national probability sample of households was selected throughout the 50 states and the District of Columbia, from each chosen household, one eligible person aged 15-44 was randomly selected for an interview Blacks, Hispanics, and teenagers were oversampled to produce accurate national estimates From March 2002 to February 2003, trained female investigators conducted in-person interviews with 4,928 men and 7,643 women Computer-assisted personal interviewing was used to gather information about fertility, contraceptive use, sources and types of family planning services, and maternal and child health Different interviews, containing gender-specific questions, were used for men and women Audio computer-assisted self-interviewing (ACASI) was used to collect the most sensitive information, including data on illicit drug use and STD risk behaviors, to give respondents privacy The overall response rates were 78% for men and 80% for women

Illicit drug use

All respondents were asked how often they had smoked marijuana, had used cocaine or crack, or had injected nonprescription drugs during the last 12 months. Response options were "never", "once or twice", "several times", "about once a month", "about once a week", and "about once a day." We defined exposure to drugs as use of one or more of these substances at least once during the previous year. Because of the relatively low prevalence of cocaine and crack use, we combined both into one group (cocaine). Data on the frequency of cocaine, crack, and injection-drug use were not included in the NSFG data file that was available for statistical analyses; therefore, we dichotomized each of the drug use measures into either "used during the last 12 months" or "did not use during the last 12 months". Respondents were considered non-users if they reported no use of any of these drugs in the last 12 months.

Outcomes

The NSFG assessed a broad range of reproductive health outcomes. Because we focused on characteristics that may directly affect pregnancy rates and outcomes, and because a small number of respondents reported same-sex sexual activities, our analyses included characteristics pertaining only to heterosexual sex. We examined data on three categories of outcomes: basic reproductive health characteristics, risky sexual behaviors, and experiences with STDs. Basic reproductive health characteristics included age at first vaginal intercourse, the lifetime number of opposite-sex partners (categorized as 0, 1, 2-4, 5-10, and 11 or more) and the number of partners in the last 12 months (categorized as 0, 1, 2, and 3 or more). For male respondents, we included information about pregnancy involvement and intention to have children (or more children). For female respondents, we examined data on parity and intention to have children (or more children). For outcomes regarding pregnancy history, we used ACASI data if available.

Four ACASI items measured specific risky sexual behaviors during the last 12 months: "Did you have sex with any females/males who were also having sex with other people at around the same time?"; "How often were you 'high' on alcohol or drugs when you had sex with a female/male?"; "Have you had sex with a female/male who takes or shoots street drugs using a needle?"; and "Has a female/male given you money or drugs to have sex with her/him?". In addition, respondents were asked whether they had used a condom at last vaginal intercourse; been tested or treated for an STD in the last 12 months; ever received a diagnosis of genital herpes, genital warts, or syphilis; and, for females, ever been treated for PID. For respondents aged 24 or younger, attitudes toward condom use were evaluated by two questions in the

personal interview: "What is the chance that if you/your partner used a condom during sex, you would feel less physical pleasure?" and "What is the chance that it would be embarrassing for you and a new partner to discuss using a condom?". The response options for both questions were "no chance", "a little chance", "a 50-50 chance", "a pretty good chance", and "an almost certain chance". We dichotomized responses into "no or little chance" and "at least a 50-50 chance".

Data on current contraceptive use (i.e. during the last three months) were available only for female respondents. This measure included only women at risk for an unintended pregnancy; women who were seeking pregnancy, pregnant, postpartum or infertile, as well as those who had not had intercourse in the previous three months, were excluded. We categorized methods into four groups, as recommended by Steiner *et al.*:[30] most effective (sterilization, implants, injectables, or IUDs), effective (pills, patches, or rings), least effective (barrier methods, natural methods, or spermicides), and no method.

Covariates
We examined differences in several demographic and socioeconomic characteristics between respondents who used and did not use drugs. From the personal interview, we included age at interview (15-19, 20-29, 30-39, and 40-44), race and ethnicity (Hispanic, non-Hispanic white, non-Hispanic black, or other), level of education (less than 12 years or 12 years or more) and whether the respondent was currently married or cohabiting. Place of residence (central city, other metropolitan area, nonmetropolitan area) was based on the respondent's address at the time of interview. The National Center for Health Statistics provided respondents' household income as a percentage of the federal poverty level (0-99%, 100-499%, or 500% or more), calculated from the total household income from all sources in the 12 months prior to the interview and census data on average threshold incomes specific to family size.[31] Data on self-reported general health status ("excellent", "very good", "good", or "fair/poor") were available from the ACASI file.

Analytic approach
We excluded the sample's 352 pregnant women from our analyses, because pregnancy may have influenced their reporting of drug use and therefore biased our results. Addiction to illicit substances is ground for termination of parental rights in some states,[32] and since it is easier to conceal illicit drug use than pregnancy, we assumed that underreporting of illicit drug use would be more common among pregnant than among non-pregnant women. Respondents with missing information on the drug use variables (35 men and 32 women) were excluded also. Thus, our analytic sample consisted of 4,893 men and 7,261 non-pregnant women. Because

this sample contained only 32 men and 22 women who had used injection drugs, their reproductive health characteristics could not be studied accurately. Therefore, we report results only for marijuana and cocaine users.

To produce prevalence estimates for demographic, socioeconomic, and basic reproductive health characteristics, drug use was classified into four mutually exclusive groups: none, marijuana use only, cocaine use only, and use of both drugs. We conducted Pearson's chi-square tests (for the categorical variables) and t-tests (for age at first vaginal intercourse, a continuous variable) to examine differences in drug use across the characteristics and reproductive health outcomes of interest.

We conducted multivariable logistic regression analyses to study the associations between marijuana and cocaine use in the last 12 months and our outcome measures while controlling for the following demographic and socioeconomic covariates: age, race and ethnicity, education, residence, and household income. In supplementary analyses, we stratified the analyses by age at interview: 25 or younger, and older than 25. We assessed statistical significance by calculating p-values in univariate analyses and 95% confidence intervals in multivariable analyses. No adjustments were made for the multiple comparisons performed. Because the NSFG used complex sampling designs, weighted analyses were necessary to adjust for different sampling rates, response rates, and coverage rates to calculate unbiased national estimates. To account for the complex sampling design, we used the Complex Samples Module in SPSS version 17.0 for Windows in all analyses.

Results

Descriptive analyses

Twenty-seven percent of men and 16% of women aged 15-44 reported drug use in the last 12 months (Table 6.1). Marijuana use was reported more frequently than cocaine use, by an estimated 25% of men and 16% of women. Prevalence of marijuana use was higher among 15-29-year-old men and women than among those aged 30-39; it was lower among Hispanics than among whites. Drug use was more commonly reported by respondents with fewer than 12 years of education than among those with 12 years or more; its prevalence was elevated among people living in a central city and among those in the lowest and highest household income categories. Smaller proportions of respondents who were married or cohabiting than of those who were not reported drug use. Overall, respondents whose health status ranged from very good to fair or poor reported drug use more frequently than did those in excellent health.

Table 6.1 Percentage distributions of U S men and non-pregnant women aged 15-44, by marijuana and cocaine use in the last 12 months, according to demographic and socioeconomic characteristics, 2002 National Survey of Family Growth [a]

Characteristic	Men					Women				
	No.	Neither	Marijuana only	Cocaine only	Both	No.	Neither	Marijuana only	Cocaine only	Both
Total	4,893	73	19	1	6	7,261	84	13	1	3
Age										
15 19 years	1,115	65***	29***	0	6	1,111	71***	25***	0	4***
20-29 years	1,634	67***	23***	2	8*	2,466	78***	18***	1	3**
30-39 years (ref)	1,460	81	12	2	6	2,493	90	8	1	2
40-44 years	684	78	18*	1	3	1,191	91	7	1	2
Race of ethnicity										
Non-Hispanic white (ref)	2,583	72	20	1	7	3,954	82	15	1	3
Non-Hispanic black	920	73	22	1	4*	1,455	85	14	0*	1*
Hispanic	1,120	77*	15**	2*	6	1,489	89***	9***	1	3
Other	270	77	16	1	6	363	83	11	1	4
Education										
<12 years	1,354	70*	23**	1	6	1,624	79***	18***	0	3
≥12 years (ref)	3,539	74	18	1	6	5,637	85	12	1	2
Place of residence										
Central city (ref)	1,878	69	22	2	7	2,731	81	16	1	3
Other metropolitan	2,281	73*	20	1*	6	3,434	86***	11***	1	2
Non-metropolitan	734	82***	14***	1	4**	1,096	83	14	0*	3
Percentage of poverty level										
0-99%	768	66***	24**	1	9**	1,522	80**	16**	1	3
100-499% (ref)	3,278	76	18	1	5	4,868	85	12	0	2
≥500%	847	69*	22	1	8*	871	81*	15	1	3
Currently married or cohabiting										
Yes (ref)	1,601	82	13	1	4	3,521	90	8	0	2
No	3,292	65***	26***	2	8***	3,740	76***	20***	1**	4***
General health										
Excellent (ref)	1,717	78	17	1	4	2,117	87	12	0	2
Very good	1,886	73**	19	1	7**	2,854	84**	14*	0	2
Good	1,022	69***	22**	2	7**	1,717	80***	15*	1*	5***
Fair/[poor	266	66**	25*	1	8*	570	85	12	1	3*

ref, reference group, * p<0 05, ** p<0 01, *** p<0 001

[a] Ns are unweighted, percentages are weighted Percentages may not add up to 100% due to rounding Differences were assessed by Pearson's chi-square test

The prevalence of drug use was higher among respondents who had ever had vaginal intercourse than among those who had not – for men, 28% vs. 18%; and for women, 17% vs. 11% (Table 6.2). Drug users had their first vaginal intercourse at a younger age than non-users (means, 15.2-16.1 vs. 17.3-17.5 years). Drug use also was far more common among respondents who had had 11 or more sexual partners of the opposite sex than among those who had had only 2-4. Its prevalence was higher among men who had had two or more partners (50%) than among those who had had one (22%) in the last year. Drug use also was more prevalent among women who had had two or more partners (36% and 46%) than among those who had had one (13%). The prevalence of reported drug use was lower among men who had been involved in a pregnancy than among men who had not (20% vs. 32%). Similarly, drug use – particularly marijuana use – was less prevalent among women who had had a live birth than among those who had not (8% vs. 21%). Some 22% of men and 18% of women who intended to have children (or more children) reported using only marijuana in the last 12 months, compared with 16% of men and 10% of women who did not. These descriptive analyses were not adjusted for age, which may have confounded the results.

Multivariable analyses

In the multivariable analyses, men and women who used drugs were as likely as non-users to have used a condom at last vaginal intercourse (Table 6.3). However, females who used marijuana or cocaine were more likely than non-users to report no current contraceptive method rather than a most effective method (odds ratio (OR) 1.5 for each). In the last 12 months, men and women who used drugs were much more likely than non-users to have had sex with a non-monogamous partner (3.3-5.2 for men and 2.9-6.5 for women), while high on alcohol or drugs (10.1-18.0 and 8.1-24.2) or in exchange for money or drugs (2.7-2.8 and 2.3-9.2). Female cocaine users were more likely than non-users to have had sex with an injection-drug user (2.8); this association did not reach statistical significance for men (1.8).

Men and women who used drugs were more likely than non-users to have been tested or treated for STDs in the last 12 months; the strongest associations were found for cocaine users. In general, drug users also were more likely than non-users to ever have received a diagnosis of genital herpes, genital warts, or syphilis (ORs 2.1-11.6 for men and 1.5-5.6 for women); the exception was that male cocaine users' lifetime prevalence of genital herpes was comparable to that of non-users. Women who used marijuana were significantly more likely than non-users to ever have received treatment for PID (1.7).

Table 6.2 Selected reproductive health characteristics of men and women aged 15-44, by marijuana and cocaine use in the last 12 months.[a]

Characteristic	No.	Neither	Marijuana only	Cocaine only	Both
MEN					
Ever had vaginal sex					
Yes	3,975	72	20	2	7
No	892	82***	16*	0**	2**
Mean age at first sex	3,945	17.3	15.8***	16.1*	15.6***
Lifetime no of female partners					
0	720	84**	14*	0	2**
1	554	84*	12*	0*	4
2-4 (ref)	1,047	77	18	1	4
5-10	1,153	72	21	2*	6
≥11	1,295	62***	25***	3**	11**
No of female partners in last 12 months					
0	1,127	81	15	1	3
1 (ref)	2,475	78	17	1	4
2	537	50***	36***	1	13***
≥3	714	50***	31***	3***	16***
Involved in ≥1 pregnancy					
Yes	1,762	80	14	1	5
No	3,131	68***	24***	1	7**
Intends to have (more) children					
Yes	3,117	70	22	1	7
No	1,739	78***	16***	1	5
WOMEN					
Ever had vaginal sex					
Yes	6,282	83	14	1	3
No	951	89**	11*	0*	1**
Mean age at first sex	6,255	17 5	15.9***	15.3***	15 2***
Lifetime no of male partners					
0	730	93***	7**	0	0*
1	1,505	91***	8***	0	1*
2-4 (ref)	2,062	85	13	0	2
5-10	1,928	81*	15	1*	3**
≥11	914	66***	24***	2***	9***
No of male partners in last 12 months					
0	1,317	90*	9	0	0*
1 (ref)	4,753	87	11	0	2
2	635	64***	27***	1***	7***
≥3	524	54***	30***	2***	13***
Parity					
0	3,037	75	21	1	4
≥1	4,181	90***	8***	1	2***
Intends to have (more) children					
Yes	3,457	79	18	1	3
No	3,705	88***	10***	1	2

ref, reference group, * p<0.05, ** p<0.01, *** p<0.001.
[a] Unless otherwise noted, data are percentages Ns are unweighted; percentages and means are weighted. Percentages may not add up to 100 because of rounding and missing data. Differences were assessed by Pearson's chi-square tests or, for mean age at first sex, t-tests

Table 6.3 Adjusted odds ratios (and 95% confidence intervals) from multivariable logistic regression analyses assessing associations between selected reproductive health outcomes and marijuana or cocaine use in the last 12 months.[a]

Outcome	Men		Women	
	Marijuana	Cocaine	Marijuana	Cocaine
Used condom at last vaginal sex[b]	0.9 (0.7-1 1)	0.8 (0 6-1 0)	1.0 (0 8-1 2)	0 9 (0 6-1 3)
Current contraceptive method[c]				
Most effective	–	–	Reference	Reference
Effective	–	–	1.0 (0 8-1.4)	1.0 (0 6-1 6)
Least effective	–	–	1.2 (0 9-1 6)	1 0 (0 6-1 6)
None	–	–	1.5 (1.1-2 2)	1 5 (1.0-2 4)
Not applicable	–	–	0 8 (0.6-1.0)	0 6 (0 4-0 9)
Risky sexual behaviors in last 12 months[d]				
Sex with non-monogamous partner	3 3 (2.5-4 2)	5.2 (3 9-7.0)	2.9 (2.3-3 7)	6.5 (4 4-9.5)
Sex while high on alcohol or drugs	10.1 (8.2-12 4)	18.0 (10.8-30 0)	8.1 (6.5-10.2)	24 2 (15.4-38.1)
Sex with injection-drug user	0 9 (0.5-1 7)	1 8 (0 9-3.7)	0 8 (0.5-1.3)	2.8 (1 2-6 5)
Received money or drugs for sex	2.7 (1.3-5.7)	2.8 (1.1-7.1)	2 3 (1 3-4 0)	9 2 (3.5-24 1)
Experience with STDs				
Tested for STD in last 12 months	2 1 (1.5-2.8)	2.5 (1.7-3 7)	2 5 (2.1-2.9)	3.6 (2 5-5.2)
Treated for STD in last 12 months	4 3 (2 7-7 0)	7 0 (3.9-12.6)	2 5 (1.8-3.5)	4.0 (2.0-8 1)
Ever had genital herpes	2 4 (1.2-4 5)	1 3 (0.5-3 1)	1 5 (1.0-2 1)	2 5 (1 5-4 0)
Ever had genital warts	2.6 (1.5-4 6)	2.1 (1.2-3 6)	2.0 (1 4-3.0)	2 6 (1.6-4 4)
Ever had syphilis	4 3 (1 9-9 6)	11 6 (3.8-35 8)	3.1 (1.0-9.7)	5 6 (1.9-16 7)
Ever treated for PID	–	–	1 7 (1 2-2 2)	1 7 (0 9-3 3)
Attitudes toward condom use[e]				
Reduce pleasure	1 5 (1 2-1.9)	1 8 (1.2-2 6)	1 0 (0.8-1 2)	1 3 (0 8-2.3)
Embarrassing to discuss with new partner	0 4 (0 3-0.6)	0 4 (0 2-0 7)	0 4 (0 3-0 6)	0 5 (0 2-1 3)

PID, pelvic inflammatory disease; STD, sexually transmitted disease.
[a] All data are weighted. Odds ratios are adjusted for age at interview, race and ethnicity, level of education, residence, and household income. All outcomes except current contraceptive method are dichotomous.
[b] Based on those who ever had vaginal sex
[c] Most effective methods are sterilization, implants, injectables, and IUDs, effective methods are pills, patches, and rings, least effective methods are barrier methods, natural methods, and spermicides
[d] Based on those who reported any opposite-sex partners in last 12 months.
[e] Assessed only among respondents younger than 25 Categories shown refer to respondents who believe there is at least a 50% chance of this outcome.

Among respondents aged 15-24, men who used drugs were more likely than non-users to think that they would feel less physical pleasure if they used a condom during sex (ORs 1.5 and 1.8 for marijuana and cocaine users, respectively). This association was not present among female respondents. However, all 15-24-year-olds who used drugs – except for female cocaine users – were less likely than non-users to think that it would be embarrassing to discuss condom use with a new partner (0.4 for each).

Age-stratified analyses

Among 15-25-year-old male respondents, marijuana and cocaine users were more likely than non-users to ever have had vaginal intercourse (84% and 93% vs. 59%), but among older men no such difference was evident (Table 6.4). Among both young and older women, those who used marijuana and cocaine were more likely than non-users to ever have had vaginal intercourse; the difference was especially pronounced among young women (85% and 94% vs. 62%). In both age-groups, male and female drug users were, on average, younger at first intercourse and had had more opposite-sex partners ever and in the last 12 months than non-users.

Table 6.4 Percentage of men and women, by age-group and by marijuana or cocaine use in the last 12 months, according to selected reproductive health characteristics [a]

Characteristic	15-25 years			26-44 years		
	Neither	Marijuana	Cocaine	Neither	Marijuana	Cocaine
MEN						
Ever had vaginal sex	59	84***	93***	95	95	97
Mean age at first sex	16 4	15 6***	15 5***	17 5	15.9***	15.9***
Lifetime no of female partners						
0	34	10***	3***	5	5	3
1	21	14**	13	11	2*	2
2-4 (ref)	25	29	24	23	11	9
5-10	13	27**	27*	31	27*	25
≥11	8	21***	32***	30	56***	62***
No of female partners in last 12 months						
0	41	16***	9***	11	11	10
1 (ref)	42	40	40	78	64	54
2	8	19***	14*	4	12***	16***
≥3	9	26***	36***	6	13***	20***
WOMEN						
Ever had vaginal sex	62	85***	94***	96	98*	99*
Mean age at first sex	16 6	15 8***	15.3***	17 8	15.7***	15.1***
Lifetime no of male partners						
0	30	8***	2***	4	1	0
1	28	18***	8**	22	5**	3
2-4 (ref)	24	35	24	32	15	10
5-10	14	26	34***	30	36***	27**
≥11	4	14***	31***	12	43***	61***
No of male partners in last 12 months						
0	37	14***	4**	11	6	3
1 (ref)	50	44	33	80	68	49
2	8	19***	21***	5	13***	23***
≥3	6	23***	42***	4	12***	26***

ref, reference group; * p<0 05, ** p<0 01, *** p<0.001.
[a] Unless otherwise noted, data are percentages. All data are weighted. Differences were assessed by Pearson's chi-square tests or, for mean age at first sex, t-tests

Young male cocaine users, but not those older than 25, were less likely than non-users to have used a condom at last vaginal intercourse (OR 0.5; Table 6.5). Among both young and older men, drug users were more likely than non-users to have had a non-monogamous partner (2.7-3.7 for young men and 3.5-6.0 for older men) and to have had sex while high on alcohol or drugs (9.6-28.2 and 12.5-14.5). In addition, older men who used marijuana and cocaine, but not their younger counterparts, were more likely than non-users to have received money or drugs for sex (2.9 and 3.9, respectively). Men in both age groups who used drugs were more likely than non-users to have been tested or treated for an STD in the last 12 months (2.1-10.8 for younger men and 2.0-3.8 for older men). Young and older male marijuana users had received diagnoses of genital warts or syphilis more often than nonusers (3.7-5.6 and 2.5-3.9), as had older cocaine users (2.1-12.5). Younger male marijuana users also received diagnoses of herpes more often than nonusers (5.6), although for older users the finding was marginal (2.0).

Table 6.5 Adjusted odds ratios (and 95% confidence intervals) from multivariable logistic regression analyses assessing associations between men's reproductive health outcomes and marijuana or cocaine use in the last 12 months, by age group.[a]

Outcome	15-25 year		26-44 years	
	Marijuana	Cocaine	Marijuana	Cocaine
Used condom at last vaginal sex[b]	0.8 (0.6-1.1)	0.5 (0.3-0.8)	0 9 (0 7-1 2)	1 0 (0 6-1 5)
Risky sexual behaviors in last 12 months[c]				
Sex with non-monogamous partner	2 7 (2.0-3.6)	3.7 (2.6-5.4)	3 5 (2.4-5 1)	6.0 (3 7-9 7)
Sex while high on alcohol or drugs	9 6 (7.1-12 9)	28 2 (14 7-54 3)	12 5 (9 0-17 3)	14 5 (7 5-28 3)
Sex with injection-drug user	0 6 (0.2-1 5)	1 0 (0.2-4.0)	1 2 (0 5-2.7)	2 3 (1 0-5.2)
Received money or drugs for sex	2 2 (0 9-5.0)	1.1 (0.2-6.0)	2.9 (1.1-7 6)	3 9 (1 2-12 4)
Experience with STDs				
Tested for STD in last 12 months	2.1 (1.5-2 8)	2.5 (1.5-4.2)	2 0 (1.2-3 4)	2 3 (1 2-4.3)
Treated for STD in last 12 months	4 6 (2.4-8.7)	10 8 (4.7-24.8)	3 8 (2 0-7 5)	3.4 (1 8-6 2)
Ever had genital herpes	5 6 (1 9-16 4)	4 4 (0 8-25.5)	2 0 (0 9-4.2)	1.0 (0 3-2.9)
Ever had genital warts	3.7 (1 2-11 1)	3 0 (0 6-15.3)	2 5 (1 4-4 7)	2.1 (1.1-4 1)
Ever had syphilis	5 6 (1 2-27 0)	—[d]	3 9 (1.7-9.2)	12.5 (3 6-43 1)

STD, sexually transmitted disease
[a] All data are weighted Odds ratios are adjusted for age at interview, race and ethnicity, level of education, residence, and household income. All outcomes are dichotomous.
[b] Based on those who ever had vaginal sex
[c] Based on those who reported any female sexual partners in last 12 months
[d] Could not be estimated reliably because of sparse data

Both young and older female drug users also exhibited patterns of sexual risk-taking and STD experiences (Table 6.6). Current contraceptive use varied little, except that young female cocaine users were more likely than non-users to be using no contraceptive method rather than a most effective method (OR 2.7). In both age-groups, women who used drugs were more likely than non-users to have had sex with a non-monogamous partner (2.8-5.3 for younger women and 2.9-7.1 for older women) or while high on alcohol or drugs (7.1-24.1 and 10.9-25.5). The odds of exchanging sex for money or drugs were higher among young users of marijuana or cocaine (3.3-12.5) than among non-users; however, among older women, only cocaine users had higher odds (7.1). Young women who used cocaine were more likely than non-users to have had a sexual partner who injected drugs (3.1). Having

Table 6.6 Adjusted odds ratios (and 95% confidence intervals) from multivariable logistic regression analyses assessing associations between women's reproductive health outcomes and marijuana or cocaine use in the last 12 months, by age group [a]

Outcome	15-25 years		26-44 years	
	Marijuana	Cocaine	Marijuana	Cocaine
Used condom at last vaginal sex[b]	0 9 (0 7-1.1)	0 7 (0 4-1.2)	0.9 (0.7-1.1)	1 1 (0 6-1 8)
Current contraceptive method[c]				
Most effective	Reference	Reference	Reference	Reference
Effective	1 2 (0 8-1 9)	1 6 (0.6-4 3)	0.8 (0.6-1.2)	0 8 (0.4-1 4)
Least effective	1 4 (0 9-2 2)	1 8 (0 8-4 2)	0 9 (0 7-1 3)	0 6 (0.3-1.0)
None	1.2 (0 7-2 1)	2.7 (1.2-5.9)	1.4 (0 8-2 2)	0 9 (0.4-1 8)
Not applicable	0.6 (0 4-0 9)	0 6 (0 2-1 8)	0.8 (0 5-1 1)	0 5 (0.2-1 0)
Risky sexual behaviors in last 12 months[d]				
Sex with non-monogamous partner	2.8 (2 1-3 8)	5 3 (3.1-9.0)	2.9 (2 1-4 2)	7.1 (3 8-13.3)
Sex while high on alcohol or drugs	7 1 (5.0-9 9)	24.1 (12.9-44.8)	10.9 (7 5-15 9)	25.5 (14.8-43 8)
Sex with injection-drug user	0 9 (0.4-1.9)	3.1 (1.1-8 8)	0.5 (0 2-1 1)	2 2 (0 6-8.1)
Received money or drugs for sex	3 3 (1.6-6.8)	12.5 (4.4-35.4)	1.5 (0 7-2.9)	7.1 (2.4-21.5)
Experience with STDs				
Tested for STD in last 12 months	2 7 (2 1-3 4)	3 4 (2 1-5.4)	2 3 (1.7-3.0)	3.8 (2.0-7 0)
Treated for STD in last 12 months	2 3 (1 5-3.8)	3 3 (1.6-6 9)	2 8 (1.8-4 3)	5.6 (2.3-13 5)
Ever had genital herpes	2 4 (1 0-5 6)	4 8 (1 6-14 1)	1.5 (1.0-2.3)	2 7 (1 4-5 1)
Ever had genital warts	4.2 (2 5-7 2)	8 2 (3 9-17 1)	1 9 (1.1-3.0)	2 0 (1 0-4 1)
Ever had syphilis	_[d]	_[d]	3 6 (1.2-11.1)	6 1 (1 9-19.0)
Ever treated for PID	1.6 (1 0-2 7)	2 0 (0 9-4 7)	1 8 (1.2-2.6)	1 8 (0 8-4 0)

PID, pelvic inflammatory disease, STD, sexually transmitted disease
[a] All data are weighted. Odds ratios are adjusted for age at interview, race and ethnicity, level of education, residence, and household income All outcomes except current contraceptive method are dichotomous.
[b] Based on those who ever had vaginal sex
[c] Based on those who reported any male partners in last 12 months
[d] Could not be estimated reliably because of sparse data

been tested or treated for STDs in the last 12 months was associated with drug use among women in both age groups (2 3-3 4 for younger women and 2 3-5 6 for older women), as was having a history of genital herpes or genital warts (2 4-8 2 and 1 5-2 7) Having had treatment for PID was associated with marijuana use for both age groups (1 6 and 1 8) A history of syphilis was strongly associated with drug use among older women (3 6 for marijuana and 6 1 for cocaine), but this association could not be estimated accurately in the younger age group because of sparse data

Discussion

Our results confirm that a substantial proportion of U S men and women of reproductive age used illicit drugs in the last year A greater proportion of marijuana and cocaine users than of non-users had unfavorable reproductive health characteristics and therefore higher odds of sexual health problems Some differences in the patterns of reproductive health characteristics appeared between the two age groups, but most associations between drug use and risky sexual behaviors and experiences with STDs occurred among both young and older men and women

Although no direct linkage could be proven, marijuana and cocaine users generally began sexual activity earlier than non-users This association suggests that the element of risk-taking may explain the link between drug use and many dangerous sexual behaviors, as has been suggested previously [13 14] Early sexual activity may lead to problems for the individual as well as society younger adolescents are less likely than older adolescents to use contraceptives at first vaginal intercourse because of a lack of sexual knowledge Thus, they are at increased risk for unintended pregnancy [33] In the United States, approximately 50% of unintended pregnancies end in an induced abortion [34] Unintended pregnancies ending in an unplanned birth are associated with an increased risk of exposure to behaviors, such as smoking and late initiation of prenatal care, that could jeopardize the health of both mother and child [35]

Compared with individuals who did not use drugs, both marijuana and cocaine users reported higher numbers of partners of the opposite sex ever and in the last 12 months, which raised their risk of acquiring an STD or a genital human papillomavirus infection Indeed, these individuals reported a higher lifetime prevalence of genital warts, suggesting an increased exposure to the human papillomavirus, [12] they also were more likely than non-users to have been tested or treated for STDs in the last year, although it is uncertain which event came first, since

the data are cross-sectional. These differences may result from drug users' higher number of sexual partners, as well as other high-risk sexual behaviors, such as exchanging sex for money or drugs. Although published data on STDs among drug users are sparse, the STD prevalence we observed among cocaine users is comparable with the rates reported by Semaan *et al.*[36]

Drug use overall was not associated with the likelihood of having used a condom at last vaginal intercourse, although young male cocaine users were less likely than young non-users to have used a condom. Males aged 15-24 who used drugs thought they would feel less physical pleasure if they used a condom during sex, which may explain the lower rates of condom use in this group. This supposition should be tackled in STD prevention programs to increase condom use. Our finding that young people who use drugs are less likely to be embarrassed discussing condom use with a new partner suggests that increasing condom use is an attainable goal. Why the odds of using no contraceptive method were elevated among young female cocaine users remains a question, but efforts should be made to reduce this difference.

Our results suggest that marijuana and cocaine use may serve as proxies for past and current sexual behaviors that increase the risk of unintended pregnancies and STDs throughout the reproductive age span. Integrating sex education into drug rehabilitation programs could help to decrease the prevalence of risky sexual behaviors among participants and lead to major public health improvements. In fact, HIV interventions in drug treatment programs have led to clinically relevant reductions in risk behaviors, especially when the intensity of the intervention has been high (i.e. practicing rather than describing condom use skills) and the intervention has been delivered near the end of drug treatment.[37 39] However, in the United States, only about half of substance abuse treatment programs exclusively for adolescents have adopted HIV risk assessment and prevention services.[40]

Future research should examine the associations between drug use and sexual risk-taking so that tailored and more effective prevention programs can be developed. Research also should target associations between drug use and reproductive health to support the prevention of problems, such as diminished fertility and negative pregnancy outcomes.

Limitations
This study has several limitations. The cross-sectional design made it impossible to determine the order of events or to address causality. Additionally, self-reported illicit drug use leads to misclassification, since some respondents falsely deny drug use for

fear of prosecution or judgment [41] However, using ACASI to collect data on drug use and other sensitive topics presumably yielded more reliable results than personal interviews would have [42] Computer-assisted modes of data collection are probably the best methods for collecting data on illicit drug use in the absence of biological sampling The reproductive health characteristics of injection-drug users could not reliably be estimated because of the low prevalence of reported use Because the NSFG did not assess medical records or clinical documentation, data on STD testing and treatment were susceptible to misclassification bias as well, although the degree of underreporting of having received STD services may have been decreased substantially through the use of ACASI [43] Finally, we could not study associations between drug use and unintended pregnancies and induced abortions, as only 60% of induced abortions were reported in the NSFG [44]

Conclusions
Many U S men and women throughout the reproductive age range use illicit drugs and the prevalence of risky sexual behaviors is elevated among those who do Thus, STD prevention programs for people who use drugs should span the reproductive years and not focus solely on adolescence or young adulthood At the same time, these programs may consider creating age-specific messages, since patterns of reproductive health characteristics, including sexual risk-taking, differed slightly by age group Interventions should target the prevention of STDs and HIV, they also should target preventing pregnancies among drug users, especially unintended pregnancies, since prenatal exposure to illicit drugs may be detrimental to the health of both mother and child

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention

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Chapter | 7

Characterstics of pregnant illicit drug users and associations between cannabis use and perinatal outcome in a population-based study

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Abstract

Background
According to the 2004 National Survey on Drug Use and Health, 4.6% of American women reported use of an illicit drug during pregnancy. Previous studies on illicit drug use during pregnancy and perinatal outcomes showed inconsistent results.

Methods
This population-based study included mothers who delivered live-born infants without birth defects between 1997 and 2004 and completed interviews for the National Birth Defects Prevention Study (response rate 69%; $n=5,871$). Prevalence of self-reported illicit drug use (specifically cannabis, cocaine, and stimulants) during pregnancy and its associations with demographic and social factors were assessed. We used multivariable linear and logistic regression analyses to study the associations of cannabis use with birth weight and gestational age.

Results
The prevalence of reported illicit drug use during pregnancy was 3.6% (standard error 0.24). Pregnant users of cannabis, cocaine, and stimulants were younger, had a lower level of education and lower household income, and were less likely to have used folic acid in the periconceptional period than non-users. Illicit drug users were also more likely to have used alcohol and tobacco. After adjustment for confounding, cannabis use was not associated with mean birth weight or gestational age or with low birth weight or preterm delivery.

Conclusion
Women who report use of illicit drugs during pregnancy differ in demographic and socioeconomic background from non-users. Reported cannabis use does not seem to be associated with low birth weight or preterm birth.

Background

In 2004, the National Survey on Drug Use and Health indicated that 4 6% of American women of 15-44 years of age reported use of an illicit substance during pregnancy [1] Studies recently conducted in the U S report even higher prevalences of perinatal illicit drug use up to 12 4% [2] A few studies have shown that pregnant cannabis and cocaine users differ in background characteristics from non-using pregnant women,[3 5] but studies using a population-based random sample of U S live births are scarce

Infants of women who used cannabis during pregnancy have been reported to have lower birth weights[2 6] and a decreased gestational age[7] compared to infants of non-users However, most studies did not find an association between cannabis use and low birth weight (LBW),[3 8] gestational age, or preterm birth [3 6] Nevertheless, several biological mechanisms by which cannabis could influence perinatal outcome have been proposed [9 10] Since children born preterm or with LBW have an increased risk of infant mortality and long-term morbidity,[11 12] identifying risk factors for these adverse outcomes is of importance

Methods

The National Birth Defects Prevention Study (NBDPS)

The NBDPS is an ongoing population-based case-control study that includes case infants with major structural congenital malformations identified via 10 birth defects surveillance systems in Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah Control infants are live-born infants without major birth defects from the same geographical areas, randomly selected from birth hospital records or birth certificates Mothers are interviewed by trained interviewers via telephone in either English or Spanish between 6 weeks and 24 months after the estimated date of delivery Questions are asked about demographic characteristics, maternal health, lifestyle factors, and occupation The methods and enrolment of the infants have been described in detail elsewhere [13 14] For this study, we selected all control infants born between October 1, 1997, and December 31, 2004 whose mothers completed the interview (*n*=5,871) The response rate was 69%

Exposure and outcome assessment

Detailed information on the type, timing, and frequency of maternal illicit drug use during the period from three months before pregnancy until birth of the index child was available from the interview We grouped the illicit substances reported by the

mothers into five drug categories (cannabis, cocaine, stimulants, hallucinogens, and opioids) as described elsewhere.[15] Non-users were defined as women who did not report use of any illicit drug from three months before pregnancy through birth of the index child.

Data on birth weight and gestational age were obtained through abstraction of birth hospital records or birth certificates depending on how the infants were selected. During the examination of these data, some reporting inconsistencies were observed (e.g., an infant of 3,104 g at 21 weeks of gestation). To address these implausible birth weight-gestational age combinations, we used the cut-points of birth weight values within the range for their specific gestational age as proposed by Alexander *et al.*[16] For the perinatal outcome analyses, infants with implausible birth weight-gestational age combinations ($n=16$), infants with missing birth weight or gestational age data ($n=20$), and mothers with multiple gestations ($n=174$) were excluded.

Statistical analyses

We used basic descriptive statistics to describe the characteristics of women who used or did not use illicit drugs during pregnancy. The characteristics of interest were maternal age at delivery, race or ethnicity, level of education, household income, employment status, prepregnancy body-mass index (BMI), gestational weight gain (women with a weight gain of >40 kg or a weight loss of >20 kg were excluded), parity, previous induced abortions, use of contraception before or during pregnancy, any periconceptional folic acid use (from one month before through the first month of pregnancy), and any use of alcohol and cigarette smoking during pregnancy as well as paternal drug use, since most of these factors are known to affect pregnancy outcome.

A priori power analyses ($\alpha=0.05$, study power 80%) showed that the prevalence of use of cocaine, stimulants, hallucinogens, and opioids was insufficient to study their effects on perinatal outcome with satisfactory statistical power. We used multivariable linear regression techniques to study the associations between cannabis use and birth weight and gestational age, in which we included the potential confounders maternal race/ethnicity (non-Hispanic white or other), level of education (0-12 years or >12 years), cigarette smoking, binge drinking (≥ 4 drinks per sitting), and maternal age, prepregnancy BMI, and gestational weight gain as linear covariates. For the birth weight analyses, we also included gestational age as a linear term. These potential confounders were selected based on a priori knowledge and exploratory data analyses, including the findings of the descriptive analyses. Potential confounders were dropped from the model when their removal did not change the effect estimate for cannabis use by more than 10%. Similarly, we used multivariable logistic

regression to study the associations between prenatal cannabis exposure and LBW (birth weight <2500 g) and preterm birth (gestational age <37 weeks), in which maternal age (<25 years or ≥25 years) and prepregnancy BMI (<18.5 kg/m² or ≥18.5 kg/m²) were categorized, because they did not show linear relationships with the outcomes. In subanalyses, we conducted stratified analyses by trimesters of use, which were not mutually exclusive since the numbers of women who only used cannabis in the second or third trimester were very small. Statistical analyses were performed using SPSS Version 16.0 for Windows (SPSS Inc., Chicago, IL).

Results

Of the 5,871 women, 277 (4.7%, standard error [SE] 0.27) reported use of an illicit drug in the 3 months before pregnancy. Illicit drug use during pregnancy was reported by 210 women (3.6%, SE 0.24). Cannabis was the most commonly used illicit drug (n=189), followed by cocaine and stimulants (n=27). Of the cocaine users, 22 women used powder cocaine, 1 woman used crack, and 4 women used a combination of both. Opioids and hallucinogens were reported by only 4 and 2 women, respectively. Most illicit drug users (84.3%) took one illicit substance, while 15.7% used two or more illicit drugs.

Women who reported use of cannabis, cocaine, or stimulants during pregnancy were on average younger than non-users (Table 7.1). Cannabis users were more often non-Hispanic black and less often Hispanic than non-users, whereas pregnant cocaine users were more often of Hispanic origin. Women who reported illicit drug use were more likely to have a low level of education, to have a household income below $20,000, or to be unemployed. They were also more often underweight (BMI<18.5 kg/m²) than women who did not report use of illicit drugs during pregnancy. Cannabis users were more likely than nonusers to have excessive weight gain during pregnancy. Women who reported use of any illicit drug were less likely to have used folic acid in the periconceptional period. In addition, cannabis users were less likely to have had children before, but more likely to have had an induced abortion in the past. A similar pattern was seen for women who reported use of stimulants, but not for women who reported use of cocaine. Illicit drug users more often reported any use of alcohol or cigarette smoking during pregnancy and far more often reported that their partners used illicit drugs.

Table 7.1 Odds ratios with 95% confidence intervals for the characteristics of cannabis, cocaine, and stimulants users during pregnancy compared with pregnant non-users Data from the National Birth Defects Prevention Study, 1997-2004

Maternal characteristics	Non-users (n=5,547)[a]		Cannabis users (n=189)[a]			Cocaine users (n=27)[a]			Stimulants users (n=27)[a]		
	No.	%	No.	%	OR (95% CI)[b]	No.	%	OR (95% CI)[b]	No.	%	OR (95% CI)[b]
Age at delivery											
<20 years	560	10 1	52	27 5	4 6 (2 9-7 3)	12	44 4	8 0 (2 6-24 9)	11	40.7	7.3 (2.3-23.1)
20-24 years	1,196	21.6	86	45.5	3.6 (2.3-5.5)	7	25 9	2.2 (0.6-7.5)	10	37 0	3 1 (1 0-10.0)
25-29 years	1,493	26 9	30	15 9	Reference	4	14 8	Reference	4	14.8	Reference
≥30 years	2,298	41 4	21	11 1	0.5 (0.3-0.8)	4	14.8	0.6 (0.2-2.6)	2	7 4	0 3 (0 1-1 8)
Race or ethnicity											
Non-Hispanic white	3,320	59 9	122	64.6	Reference	10	37.0	Reference	17	63 0	Reference
Non-Hispanic black	623	11.2	33	17 5	1.4 (1.0-2.1)	3	11.1	1 6 (0.4-5.8)	1	3.7	–
Hispanic	1,263	22 8	29	15 3	0 6 (0.4-0 9)	14	51.9	3.7 (1 6-8.3)	6	22.2	0.9 (0.4-2 4)
Other	320	5 8	5	2.6	0.4 (0.2-1 0)	0	0 0	–	3	11 1	1 8 (0 5-6 3)
Education ≤12 years	2,249	40 6	130	68 8	3.2 (2.4-4 4)	21	77 8	5 1 (2 1-12 7)	22	81.5	6 4 (2.4-17.0)
Household income <$20,000	1,515	27 3	105	55 6	3.6 (2.6-4 9)	17	63.0	4 8 (2 1-11.2)	16	59 3	3 6 (1 6-8 0)
Employment status											
Employed	3,980	71 8	141	74 6	Reference	17	63 0	Reference	15	55.6	Reference
Unemployed	47	0 8	6	3 2	3.6 (1.5-8 6)	2	7.4	10 0 (2 2-44.3)	2	7 4	11 3 (2 5-50 8)
Other[c]	1,502	27 1	41	21 7	0 8 (0 5-1 1)	8	29.6	1 2 (0 5-2.9)	10	37.0	1.8 (0.8-3.9)
Prepregnancy BMI											
Underweight (<18 5 kg/m²)	284	5 1	21	11 1	1.9 (1 2-2.9)	7	25.9	5.3 (2.1-13.2)	5	18.5	3 5 (1 3-9 7)
Normal weight (18 5-24.9 kg/m²)	2,991	53 9	115	60 8	Reference	14	51 9	Reference	15	55.6	Reference
Overweight (25 0-29 9 kg/m²)	1,194	21.5	23	12 2	0.5 (0.3-0 8)	5	18.5	0 9 (0.3-2 5)	5	18 5	0.8 (0 3-2 3)
Obese (≥30.0 kg/m²)	850	15 3	28	14 8	0.9 (0.6-1.3)	1	3.7	–	2	7.4	0 5 (0.1-2.0)
Gestational weight gain[d]											
Weight loss or limited weight gain (≤11 5 kg)	1,910	34 4	51	27 0	1.0 (0 7-1 6)	7	25.9	0.8 (0 3-2.4)	6	22.2	0.8 (0.3-2 6)
Appropriate weight gain (11.6-16.0 kg)	1,595	28.8	41	21 7	Reference	7	25 9	Reference	6	22 2	Reference
Excessive weight gain (16.1-40.0 kg)	1,911	34.5	93	49.2	1.9 (1.3-2.8)	12	44.4	1.4 (0.6-3.6)	13	48.1	1 8 (0.7-4 8)
Parity ≥1	3,378	60 9	78	41 3	0.5 (0.3-0.6)	14	51.9	0.7 (0.3-1.5)	9	33 3	0 3 (0.1-0 7)
Induced abortions ≥1	696	12.5	41	21.8	1.9 (1.4-2 8)	5	18.5	1 6 (0 6-4.2)	6	22 2	2.0 (0.8-5.0)
Use of contraception	1,561	28.1	60	31.7	1.2 (0.9-1 6)	7	25 9	0 9 (0 4-2 1)	7	25 9	0 9 (0 4-2 1)
Periconceptional use of folic acid	2,882	52.0	58	30.7	0.4 (0 3-0.6)	5	18.5	0 2 (0.1-0 6)	7	25 9	0 3 (0.1-0.8)

Table 7.1 (Continued)

Maternal characteristics	Non-users (n=5,547)[a]		Cannabis users (n=189)[a]			Cocaine users (n=27)[a]			Stimulants users (n=27)[a]		
	No.	%	No.	%	OR (95% CI)[b]	No.	%	OR (95% CI)[b]	No.	%	OR (95% CI)[b]
Any alcohol use or cigarette smoking during pregnancy											
No alcohol used, no cigarette smoking	3 615	65.2	22	11.6	Reference	2	7 4	Reference	2	7.4	Reference
Alcohol used, no cigarette smoking	1,110	20.0	31	16 4	4.6 (2 6-8 0)	9	33.3	14 7 (3 2-67 9)	2	7.4	3 3 (0 5-23 1)
No alcohol used, smoked cigarettes	450	8.1	45	23 8	16.4 (9.8-27 6)	4	14 8	16.1 (2.9-88 0)	5	18 5	20.1 (3.9-104)
Alcohol used and smoked cigarettes	351	6 3	91	48.1	42 6 (26 4-68.7)	12	44.4	61 8 (13 8-277)	18	66.7	92.7 (21 4-401)
Paternal use of											
Cannabis	274	4.9	141	74 6	69 9 (47.9-102)	12	44 4	25 1 (10 5-60 1)	17	63 0	45 7 (18 8-111)
Cocaine	42	0 8	20	10 6	16 2 (9 3-28.2)	10	37.0	99 5 (41 3-239)	4	14.8	27 2 (8 9-83 4)
Stimulants	17	0.3	18	9.5	35 4 (17 9-70 0)	3	11.1	50 7 (13 7-187)	17	63.0	909 (320-2587)

[a] Numbers do not add up to total group size due to missing values

[b] Odds ratio with 95% confidence intervals.

[c] Other homemaker/parent, student, or disabled

[d] Classification is the recommendation for a woman with a normal prepregnancy BMI [17]

Table 7.2 Effects of prenatal cannabis exposure on birth weight and gestational age and on the occurrence of low birth weight and preterm birth stratified by cigarette smoking status Data from the National Birth Defects Prevention Study, 1997-2004.

Drug group	Number of cannabis		Birth weight	Low birth weight			Gestational age	Preterm birth		
			β (95% CI)[a]	Number (%) of cases		OR (95% CI)	β (95% CI)[a]	Number (%) of cases		OR (95% CI)[b]
	Users	Non-users		Exposed	Non-exposed			Exposed	Non-exposed	
Any cannabis use	185	5,343	-17 (-90-56)[b]	9 (4.9)	243 (4 5)	0 7 (0.3-1 6)[c]	-0.1 (-0 4-0.3)[d]	18 (9 7)	410 (7.7)	1.0 (0 6-1 9)[e]
Non cigarette smokers	51	4,557	-31 (-164-101)[f]	1 (2.0)	189 (4.1)	–	0.2 (-0.3-0.7)	3 (5 9)	335 (7.4)	0.6 (0 1-2 4)[g]
Cigarette smokers	134	785	-14 (-102-75)[f]	8 (6.0)	54 (6.9)	0.7 (0.3-2 0)[h]	-0 2 (-0.6-0.3)[i]	15 (11 2)	75 (9 6)	1 2 (0.7-2.1)
1ˢᵗ trimester cannabis use	174	5,343	-5 (-81-72)[i]	9 (5.2)	243 (4.5)	0 7 (0 3-1.7)[c]	-0 1 (0 4-0.2)[k]	17 (9.8)	410 (7.7)	1 1 (0 6-1 9)[i]
Non cigarette smokers	48	4,557	-9 (-150-131)[m]	1 (2.1)	189 (4.1)	–	0 2 (-0.3-0.8)	3 (6 2)	335 (7 4)	0 6 (0.1-2 6)[g]
Cigarette smokers	126	785	-4 (-95-86)[n]	8 (6.3)	54 (6 9)	0 7 (0 2-2.1)[o]	-0 2 (-0.7-0 2)[p]	14 (11.1)	75 (9 6)	1 2 (0 6-2.2)
2ⁿᵈ trimester cannabis use	76	5,343	-100 (-202-1)[q]	6 (7 9)	243 (4.5)	0 9 (0 3-2.8)[c]	-0 4 (-0 9-0 1)[e]	11 (14.5)	410 (7.7)	1 6 (0 8-3 3)[i]
Non cigarette smokers	19	4,557	-41 (-257-175)[s]	1 (5 3)	158 (4.1)	–	-0 4 (-1.2-0 4)[g]	3 (15.8)	335 (7.4)	1.6 (0 4-7 2)[g]
Cigarette smokers	57	785	-136 (253—18)[t]	5 (8 8)	54 (6.9)	1.0 (0 3-3.6)[h]	-0.3 (-1.0-0 3)[v]	8 (14.0)	75 (9 6)	1 5 (0.7-3.3)[v]
3ʳᵈ trimester cannabis use	53	5,343	-89 (-209-30)[q]	4 (7.5)	243 (4 5)	0 9 (0 2-4.3)[w]	-0 5 (-1.1-0.1)[e]	8 (15.1)	410 (7 7)	1 8 (0.9-4.0)[i]
Non cigarette smokers	16	4,557	-99 (-316-118)[x]	1 (6 2)	189 (4 1)	–	-0 5 (-1 4-0 4)[g]	3 (18.8)	335 (7 4)	2.0 (0 4-9.0)[g]
Cigarette smokers	37	785	-87 (-233-59)[y]	3 (8 1)	54 (6 9)	0 8 (0 1-4.7)[h]	-0.5 (-1.3-0.3)[v]	5 (13 5)	75 (9 6)	1.8 (0.6-5.5)[v]

[a] Regression coefficient, which represents the difference in birth weight (g) or gestational age (weeks) between exposed and non-exposed infants, with 95% confidence interval

[b] Adjusted for gestational age, maternal age at delivery, race or ethnicity, cigarette smoking, binge drinking (≥4 drinks per sitting), prepregnancy BMI, and gestational weight gain.

[c] Adjusted for gestational age and cigarette smoking.

[d] Adjusted for maternal age at delivery, race or ethnicity, cigarette smoking, binge drinking, prepregnancy BMI, and gestational weight gain.

[e] Adjusted for cigarette smoking, binge drinking, and gestational weight gain.

[f] Adjusted for gestational age, maternal age at delivery, race or ethnicity, binge drinking, prepregnancy BMI, and gestational weight gain

[g] Adjusted for gestational weight gain

[h] Adjusted for gestational age

[i] Adjusted for maternal age at delivery, race or ethnicity, binge drinking, prepregnancy BMI, and gestational weight gain

Adjusted for gestational age, maternal age at delivery, race or ethnicity, cigarette smoking, binge drinking, and gestational weight gain

[k] Adjusted for cigarette smoking and binge drinking.

[l] Adjusted for cigarette smoking.

[m] Adjusted for gestational age, maternal age at delivery, race or ethnicity, binge drinking, and gestational weight gain

[n] Adjusted for gestational age, maternal age at delivery, race or ethnicity, level of education, binge drinking, prepregnancy BMI, and gestational weight gain

[o] Adjusted for gestational age and binge drinking

[p] Adjusted for binge drinking, prepregnancy BMI, and gestational weight gain

[q] Adjusted for gestational age, maternal age at delivery, and cigarette smoking.

[r] Adjusted for cigarette smoking and gestational weight gain.

[s] Adjusted for gestational age, maternal age at delivery, binge drinking, prepregnancy BMI, and gestational weight gain.

[t] Adjusted for gestational age, maternal age at delivery, and gestational weight gain.

[u] Adjusted for binge drinking

[v] Adjusted for prepregnancy BMI

[w] Adjusted for gestational age, cigarette smoking, and binge drinking.

[x] Adjusted for gestational age, maternal age at delivery, race or ethnicity, and prepregnancy BMI.

[y] Adjusted for gestational age and maternal age at delivery.

We included 5,661 infants in the analyses of the associations between cannabis use and perinatal outcomes. After adjustment for confounding factors, there was no difference in mean birth weight (-17 g, $p=0.65$) or gestational age (-0.1 weeks, $p=0.75$) between cannabis-exposed and non-exposed infants (Table 7.2). No associations between cannabis use and LBW (adjusted odds ratio [OR] 0.7, 95% confidence interval [CI] 0.3-1.6) or preterm birth (OR 1.0, 95% CI 0.6-1.9) were found either. Stratification by trimester of use did not alter these results greatly, although cannabis use during the second trimester, especially among cigarette smokers, seemed to have a detrimental effect on birth weight. In addition, the risks of preterm birth seemed slightly increased among women who used cannabis in the second (OR 1.6, 95% CI 0.8-3.3) or third trimester (OR 1.8, 95% CI 0.9-4.0). We did not detect a dose-response effect of prenatal cannabis exposure on perinatal outcome (data not shown).

Discussion

In our study, women who reported using cannabis, cocaine, or stimulants during pregnancy were similar to one another, but different from other pregnant women in a number of demographic and lifestyle characteristics. In general, prenatal cannabis use did not seem to be associated with infant birth weight or gestational age. Although we adjusted for a broad range of confounders, residual confounding by factors that we were unable to measure remains possible.

The use of illicit substances during pregnancy is likely underestimated because respondents often falsely deny use for fear of judgment or prosecution or because of feelings of shame and guilt. Previous studies have shown that 18-34% of participants who test positive through toxicological screening were missed when a questionnaire was used.[18 20] Therefore, misclassification of the exposure status of study infants has occurred, but this is most likely non-differential, especially since birth weight and gestational age were not the primary outcomes of interest in the NBDPS and evidence for recall bias among case-control studies of pregnancy outcome is scarce. Non-differential misclassification may have resulted in underestimation of exposure frequencies and less precise estimates. However, the possibility of differential misclassification of prenatal illicit drug exposure status cannot completely be excluded.

In our study, women who reported cannabis and cocaine use during pregnancy had similar characteristics as those previously reported in the literature. However, there were some discrepancies, such as the lower level of education for cannabis users, the younger maternal age of cocaine users, and the fact that the majority of cocaine

users were Hispanic as opposed to African American [4 6 21] Differences in selection and participation of the various study populations may explain these differences and our lower prevalence rates The fact that pregnant stimulant users are very similar to pregnant cannabis users has not been reported before

In the U S in 2001, the prevalences of LBW and preterm birth were 7 7% and 11 9%, respectively,[22] which is higher than those for LBW (4 7%) and preterm birth (7 9%) in our study population This difference could be due to the fact that vital statistics data, in contrast with our study population, include children with birth defects who are often born preterm,[23] but it could also be due to some selection in our population A recent study showed that the NBDPS control participants, who constitute our study population, are generally representative of their base populations [14] Our findings suggest that prenatal cannabis use overall is not associated with birth weight or gestational age, which is consistent with previous studies [3 6 8] However, cannabis use in later stages of pregnancy might have some detrimental effect on perinatal outcome

Further research is needed to determine the true association between illicit drug use and perinatal outcome, in which other approaches, such as blood, urine, or meconium analyses, might be used to assess exposure status Furthermore, it remains uncertain whether prenatal cannabis exposure as well as exposure to other illicit drugs affects the occurrence of birth defects and developmental problems later in life

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Disclaimer
The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention

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Chapter 8

Maternal periconceptional illicit drug use and the risk of congenital malformations

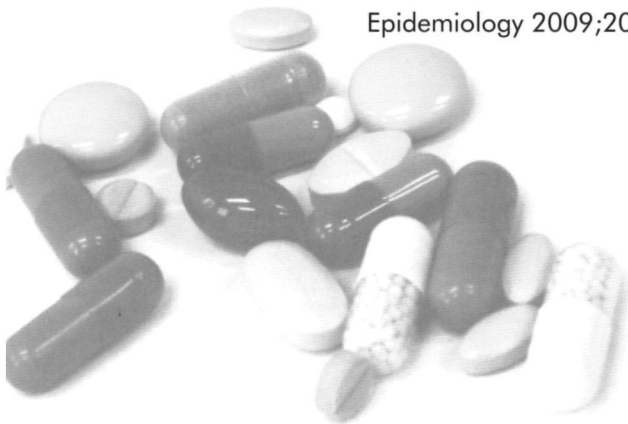Marleen M.H.J. van Gelder
Jennita Reefhuis
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Martha M. Werler
Charlotte M. Druschel
Nel Roeleveld

Abstract

Background

In 2004, the Survey on Drug Use and Health showed that 5% of American women reported use of an illicit drug during pregnancy. The results of studies determining the association between periconceptional illicit drug use and birth defects have been inconsistent.

Methods

We analyzed data from the National Birth Defects Prevention Study, a case-control study of major birth defects, and assessed all birth defects categories in which there were at least 250 interviewed case mothers. We included 10,241 infants with major congenital malformations (case infants) and 4,967 infants without major congenital malformations (control infants) born between 1997 and 2003 for whom there was a completed maternal interview with detailed information on prenatal illicit drug use and potential confounders. We used multivariable logistic regression to estimate the associations between cannabis, cocaine, and stimulant use in the month before pregnancy or during the first trimester (periconceptional period) and the occurrence of selected birth defects.

Results

In the periconceptional period, 5% of the 15,208 mothers reported any use of illicit drugs. We did not find associations between illicit drug use and most of the 20 eligible categories of congenital malformations. Periconceptional cannabis use seemed to be associated with an increased risk of anencephaly (adjusted odds ratio 1.7, 95% confidence interval 0.9-3.4), whereas cocaine use in the periconceptional period was associated with the risk of cleft palate (2.5, 1.1-5.4).

Conclusions

There were very few suggestions of positive associations between periconceptional illicit drug use and the 20 birth defects categories.

Background

In the National Household Survey on Drug Use and Health 2003-2004, 10% of American women aged 15-44 years reported use of an illicit drug in the past month [1] Of pregnant women in the same age group, 4 6% reported any illicit drug use, 3 6% reported cannabis use, and 0 3% cocaine use Studies recently conducted in the United States report even higher prevalences of prenatal illicit substance use, ranging from 6 2% to 12 4% [2 5] Therefore, many births may potentially be affected by illicit drug use, not only in the United States, but also in other countries

The results from studies assessing the relationship between prenatal illicit drug use and birth defects have been inconsistent In general, cannabis does not seem to be associated with major congenital anomalies [6 8] However, Williams *et al*[9] found an increased risk of isolated simple ventricular septal defects after prenatal marijuana use, and Torfs *et al*[10] reported an increased risk of gastroschisis in the offspring of marijuana users Periconceptional cocaine use has been associated with cardiovascular abnormalities,[11 12] gastroschisis,[13] limb defects,[14] and genitourinary tract anomalies [15 16] The relationship between other types of illicit drugs (e g , stimulants and opioids) and major birth defects has not been studied for specific defects Also, timing of exposure has not been taken into account

Several biologic mechanisms for the role of prenatal illicit drug use in the pathogenesis of major birth defects have been proposed One of the most important components in marijuana smoke is carbon monoxide, which is a known teratogen in animal models [17 18] A recent study indicated that delta-9-tetrahydrocannabinol (Δ^9-THC), the most psychoactive agent in marijuana, modulates genes that encode for growth, cell morphology, ion exchange pathways, and apoptosis in placental development [19] Human and animal studies have suggested that maternal cocaine use might affect embryonic and fetal development through vasoconstriction in maternal and fetal tissues, leading to hypoperfusion and hypoxia [14 20 21]

Determining the true associations between illicit drug use and congenital malformations is difficult because illicit drug use is commonly accompanied by other factors that can affect pregnancy outcome, such as smoking, use of alcohol, and poor prenatal care In this study, we used data from the National Birth Defects Prevention Study to investigate the relationship between periconceptional illicit drug use and selected major birth defects, while controlling for the effects of potentially confounding behavioral factors when possible

Methods

The National Birth Defects Prevention Study is an ongoing, population-based, case-control study designed to evaluate environmental and genetic risk factors for major congenital malformations. Eligible case infants were identified from birth defects surveillance systems in Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah. Case records were reviewed by clinical geneticists in each of the centers to determine initial study eligibility, and all infants with a specific defect were reviewed by one clinical geneticist before analyses to ensure consistency across sites and to assess whether case infants had multiple major defects in different organ systems or whether the case infants' defect was isolated (i.e. no additional major unrelated birth defects).[22] Control infants, live-born infants without major congenital malformations, were randomly selected from birth certificates or hospital records from the same geographic regions. All mothers of the infants were interviewed by telephone by trained interviewers in either English or Spanish by using a standardized questionnaire between 6 weeks and 24 months after the estimated date of delivery. Questions were asked about demographic characteristics, lifestyle factors, maternal health, and occupational exposures. The enrolment of case and control infants and the methods of the National Birth Defects Prevention Study have been described in detail elsewhere.[23] The participation rates for mothers of case and control infants were 71% and 67%, respectively.

For our analyses, we included case and control infants born from 1 October 1997 through 31 December 2003, whose mothers completed the entire interview. For power purposes, we limited the analyses to birth defects categories in which there were at least 250 cases with completed maternal interviews. A total of 20 birth defects categories met this criterion, including neural tube defects, several congenital heart defects, oral clefts, and certain gastrointestinal defects.

Detailed information on the type, timing, and frequency of reported maternal illicit drug use was available from the questionnaire. We grouped the illicit substances into five drug categories, which were largely based on the classification scheme of the National Institute on Drug Abuse.[24] Marijuana and hashish were included in the cannabis group. The cocaine group consisted of cocaine and crack cocaine. Amphetamine, methylenedioxymethamphetamine (MDMA or 'ecstasy'), and methamphetamine formed the stimulants group. Lysergic acid diethylamide (LSD or 'acid'), psilocybin (hallucinogenic mushrooms), and phencyclidine HCl (PCP or 'angel dust') were included in the hallucinogens group. The opioids group consisted of diacetylmorphine (heroin), oxycodone HCl, hydrocodone bitartrate, and methadone. Medical use of marijuana or methadone was included as exposure to cannabis or opioids, respectively. We defined an infant as exposed for a specific illicit drug

category if the mother reported use of one or more substances included in that illicit drug group at any time during the period starting one month before pregnancy to the end of the third month of pregnancy (periconceptional period). Unexposed infants were case and control infants whose mothers did not report use of any illicit drug in the three months prior to and during the entire index pregnancy.

Too few infants were exposed to hallucinogens and opioids to estimate the risks of congenital malformations. Infants born to women with pre-existing diabetes type 1 or type 2 ($n=220$) were excluded from the analyses because of the known strong association between this condition and congenital malformations. After exploratory data analyses, we used multivariable logistic regression techniques to study the associations between periconceptional illicit drug use and the selected birth defects. Based on a priori knowledge and the exploratory analyses, we decided to use the same potential confounder set in all models, except when small numbers prevented us from including one or more covariates. These maternal confounders were age at delivery, race or ethnicity, level of education, smoking in the periconceptional period, binge drinking (defined as ≥4 drinks per episode) in the periconceptional period, prepregnancy body-mass index (BMI), and any periconceptional folic acid use. Age at delivery and BMI were used as continuous covariates, unless the relationship between these variables and the defect studied (the natural logarithm of the odds of having a child with the specific birth defect) was not linear; in such cases, age at delivery and BMI were categorized in the analyses of these specific birth defects. The other covariates were added as dichotomous variables, with race or ethnicity categorized as non-Hispanic white or other, and level of education as 0-12 years or 13 years or more. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for periconceptional use of the particular illicit drug category if there were at least three exposed cases. When an association was found between an illicit drug category and a specific birth defect, the exposure time window was limited to the etiologically relevant period for that specific birth defect to explore the association further. In subanalyses, we excluded case and control infants who had a first-degree relative with the specific defect that was analyzed. We also conducted stratified analyses for single and multidrug cannabis users and for the frequency of periconceptional cannabis use (incidental use: ≤1 time per week; moderate use: >1 time per week, but <1 time per day; heavy use: ≥1 time per day). All statistical analyses were performed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

A total of 10,241 case infants with selected congenital malformations and 4,967 control infants were included in this study. Maternal characteristics for case and control infants are shown in Table 8.1. In general, case and control infants were comparable regarding the maternal characteristics. Slight differences were seen in race or ethnicity and in household income between case and control mothers. Furthermore, control mothers were less likely than case mothers to have 0-12 years of education, to have pre-existing diabetes, to be obese before pregnancy, and to smoke in the periconceptional period.

Table 8.1 Maternal characteristics of infants with no major birth defects and case infants with selected birth defects.[a] Data from the National Birth Defects Prevention Study, 1997-2003.

Maternal characteristics	Controls (n=4,967)		Cases (n=10,241)	
	No.	%	No.	%
Age at delivery				
<20 years	552	11 1	1,140	11 1
20-24 years	1,105	22 2	2,344	22.9
25-29 years	1,293	26.0	2,548	24.9
30-34 years	1,328	26 7	2,587	25.3
≥35 years	689	13 9	1,622	15 8
Race or ethnicity				
Non-Hispanic White	2,995	60.4	6,269	61 3
Non-Hispanic Black	580	11 7	1,022	10 0
Hispanic	1,114	22.5	2,353	23 0
Other	266	5.4	576	5 6
Education ≤12 years	2,072	41.8	4,527	44 2
Household income below median[b]	2,478	56 3	5,469	58 1
Prepregnancy BMI[c]				
Underweight	284	6.0	574	5 8
Normal weight	2,695	56 5	5,265	53.3
Overweight	1,055	22.1	2,232	22.6
Obese	734	15 4	1,801	18 2
Pre-existing diabetes type 1 or 2	25	0 5	195	1 9
Periconceptional folic acid used	2,512	50.6	5,242	51.2
Smoked in periconceptional period[d]	962	19 4	2,223	21 7
Alcohol in periconceptional period[d]	1,874	37 9	3,902	38 3
Binge drinking in periconceptional period[d]	642	13 0	1,345	13 2
Illicit drug use in periconceptional period[d]	214	4 3	483	4 7
Cannabis	190	3.8	420	4 1
Cocaine	28	0 6	77	0 8
Stimulants	28	0.6	58	0 6
Hallucinogens	3	0 1	13	0 1
Opioids	3	0.1	9	0.1

[a] Only case infants with birth defects that were classified in a category with at least 250 cases with completed maternal interviews were included
[b] Median income $40,000
[c] Body-mass index, classification of the National Institutes of Health underweight: <18 5 kg/m², normal weight 18 5-24.9 kg/m², overweight 25 0-29 9 kg/m², obese ≥30 kg/m²
[d] Periconceptional period. one month before pregnancy to the end of the third month of pregnancy

In the last month before pregnancy and during the first trimester, 4.6% of all mothers reported use of an illicit drug: 4.7% of the case mothers and 4.3% of the control mothers. Only 8 mothers refused to answer the illicit drug use questions. Cannabis was the most frequently reported illicit substance (88%), followed by cocaine (15%), and stimulants (15%). Hallucinogen and opioid use were each reported by 2% of the women who reported illicit drug use. The majority of pregnant illicit drug users (84%) used illicit drugs from one substance category. A total of 112 (16%) women used illicit drugs from two or more different categories. We did not identify a pattern in the types of congenital anomalies in the 15 infants (13 case and 2 control) who were exposed to three or more illicit drugs in the periconceptional period.

The crude and adjusted ORs for periconceptional cannabis use and the selected congenital malformations are shown in Table 8.2. Periconceptional cannabis use seemed to be associated with an increased risk of anencephaly (adjusted OR

Table 8.2 Odds ratios and 95% confidence intervals (CIs) for the association between periconceptional cannabis use and selected birth defects Data from the National Birth Defects Prevention Study, 1997-2003.

Defect	Total[a]	Cannabis exposed		Odds ratio (95% CI)	
		No.	%	Crude	Adjusted[b]
None (controls)	4,866	189	3.9	Reference	Reference
Anencephaly, craniorachischisis	244	12	4 9	1 3 (0 7-2.3)	1 7 (0 9-3 4)
Spina bifida	525	20	3 8	1 0 (0 6-1.6)	1 0 (0 6-1 6)
Anotia, microtia	287	11	3.8	1.0 (0 5-1.8)	1.0 (0.5-2 0)
Dextrotransposition of the great arteries	336	9	2.7	0.7 (0 3-1.3)	0 7 (0 3-1 4)
Tetralogy of Fallot	486	19	3 9	1 0 (0 6-1 6)	1.1 (0.6-1 8)
Hypoplastic left heart syndrome	247	7	2.8	0.7 (0.3-1.6)	0.7 (0.3-1 6)
Coarctation of aorta	433	15	3 5	0.9 (0.5-1.5)	1 0 (0.6-1 8)
Pulmonary valve stenosis	582	24	4 1	1.1 (0.7-1.7)	1 2 (0 8-1 9)
Perimembranous VSD	927	34	3 7	0 9 (0.6-1 4)	0 9 (0.6-1 4)
ASD secundum	943	31	3.3	0.8 (0 6-1 2)	0 7 (0 5-1 0)
ASD not otherwise specified	288	14	4 9	1.3 (0.7-2.2)	1 2 (0.7-2 2)
Cleft lip ± cleft palate	1,269	61	4.8	1.2 (0.9-1 7)	1 0 (0 7-1 4)
Cleft palate	677	25	3.7	0.9 (0.6-1 4)	0 8 (0 5-1 3)
Esophageal atresia ± tracheoesophageal fistula	329	12	3.6	0.9 (0.5-1.7)	1 2 (0.6-2 2)
Anorectal atresia	468	13	2 8	0.7 (0.4-1 3)	0 7 (0 4-1 2)
Hypospadias[c]	924	20	2 2	0.5 (0.3-0.8)	0 7 (0.4-1.2)
Transverse limb deficiency	315	14	4 4	1.2 (0.7-2 0)	1 1 (0.6-2 0)
Craniosynostosis	517	16	3.1	0.8 (0.5-1 3)	1 0 (0 5-1 7)
Diaphragmatic hernia	365	19	5 2	1.4 (0.8-2 2)	1 3 (0 8-2 2)
Gastroschisis	485	62	12 8	3.6 (2.7-4 9)	1 3 (0.9-1 8)

ASD, atrial septal defect; VSD, ventricular septal defect
[a] Infants born to women with pre-existing diabetes type 1 or type 2 were excluded
[b] Adjusted for maternal factors age at delivery, race or ethnicity, level of education, cigarette smoking, binge drinking, prepregnancy body-mass index, and periconceptional folic acid use
[c] Only male control infants included (n=2,452; 4.1% exposed).

1.7,95% CI 0.9-3.4). Restricting the analysis to cannabis use in the first month after conception, during which the neural tube closes, confirmed this finding (adjusted OR 2.5, 95% CI 1.3-4.9). Cannabis use in the other months of the periconceptional period was not associated with an increased risk of anencephaly. Analyses restricted to infants without a positive family history for the specific defects or to infants with isolated defects only did not alter these results. No pattern of increasing or decreasing ORs could be detected after stratification for frequency of periconceptional cannabis use, and we did not find any substantial differences in the crude ORs for the selected congenital malformations between women who used only cannabis and women who used cannabis and at least one other illicit substance (data not shown).

Because of the small numbers of infants exposed to cocaine and stimulants, we were not able to calculate adjusted ORs for all of the selected birth defects, or we could do so only with a reduced confounder set (Tables 8.3 and 8.4). The risk of spina bifida seemed to be increased after periconceptional cocaine use (adjusted OR 2.2, 95% CI 0.9-5.4), but we did not see an increased risk for use in the first month after conception, during which the neural tube closes. We observed, however, an increased odds of having a child with cleft palate among women who used cocaine in the periconceptional period (adjusted OR 2.5, 95% CI 1.1-5.4). For cocaine use in the third month after conception, during which the two palatine shelves fuse with each other, we found an adjusted OR of 6.8 (2.0-23), which was much stronger than the OR estimates for cocaine use in the other months of the periconceptional period. We did not find any increased or decreased ORs for the selected birth defects among stimulant users.

We observed increased crude ORs for having a child with gastroschisis for women with periconceptional use of cannabis, cocaine, and stimulants. However, maternal age at delivery was a strong confounder in these estimates, and the adjusted ORs (cannabis: OR 1.3 [0.9-1.8]; cocaine: OR 1.0 [0.4-2.4]; stimulants: OR 1.0 [0.5-2.3]) showed no association between illicit drug use and gastroschisis.

Discussion

Because very few previously conducted studies had sufficient numbers to look at individual birth defects and illicit drug use, this was primarily a hypothesis-generating study. We did not find associations between periconceptional cannabis, cocaine, and stimulant use and the majority of the congenital malformations assessed. However, there were possible associations between periconceptional cannabis use and anencephaly, and between cocaine use and cleft palate.

Table 8.3 Odds ratios and 95% confidence intervals (CIs) for the association between periconceptional cocaine use and selected birth defects. Data from the National Birth Defects Prevention Study, 1997-2003.

Defect	Total[a]	Cocaine exposed		Odds ratio (95% CI)	
		No.	%	Crude	Adjusted
None (controls)	4,705	28	0.6	Reference	Reference
Anencephaly, craniorachischisis	234	2	0.9	1.4 (0 3-6 1)	–
Spina bifida	512	7	1.4	2 3 (1 0-5 3)	2 2 (0.9-5 4)[b]
Anotia, microtia	279	3	1 1	1 8 (0 5-6 0)	1 8 (0.5-6 2)[c]
Dextrotransposition of the great arteries	328	1	0.3	0.5 (0 1-3 8)	–
Tetralogy of Fallot	472	5	1.1	1.8 (0.7-4 7)	1.8 (0 7-4 9)[b]
Hypoplastic left heart syndrome	240	0	0 0	–	–
Coarctation of aorta	419	1	0 2	0.4 (0.1-2.9)	–
Pulmonary valve stenosis	561	3	0.5	1 0 (0 3-3.3)	1.2 (0.4-4 1)[d]
Perimembranous VSD	902	9	1.0	1.7 (0.8-3 6)	1 4 (0 6-3 2)[b]
ASD secundum	920	8	0.9	1.5 (0.7-3.2)	1 1 (0 5-2 5)[b]
ASD not otherwise specified	275	1	0.4	0 6 (0 1-4 5)	–
Cleft lip ± cleft palate	1,216	8	0.7	1.1 (0.5-2 4)	0 9 (0 4-2.1)[b]
Cleft palate	661	9	1 4	2.2 (1.1-4.8)	2 5 (1.1-5.4)[e]
Esophageal atresia ± tracheoesophageal fistula	320	3	0 9	1.6 (0.5-5.2)	2 0 (0 6-6.7)[f]
Anorectal atresia	456	1	0.2	0.4 (0.1-2.7)	–
Hypospadias[g]	907	3	0 3	0.6 (0.2-2.1)	0.9 (0 2-3.2)[h]
Transverse limb deficiency	303	2	0 7	1.1 (0.3-4 7)	–
Craniosynostosis	505	4	0.8	1.3 (0.5-3.8)	1.8 (0 6-5.4)[i]
Diaphragmatic hernia	349	3	0.9	1.4 (0.4-4 8)	1.5 (0 4-4.8)[i]
Gastroschisis	432	9	2 1	3.6 (1.7-7 6)	1 0 (0 4-2.4)[b]

ASD, atrial septal defect; VSD, ventricular septal defect.
[a] Infants born to women with pre-existing diabetes type 1 or type 2 were excluded.
[b] Adjusted for maternal factors: age at delivery, race or ethnicity, level of education, cigarette smoking, binge drinking, prepregnancy body-mass index (BMI), and periconceptional folic acid use
[c] Adjusted for maternal factors: age at delivery, binge drinking, prepregnancy BMI, and periconceptional folic acid use.
[d] Adjusted for maternal factors: age at delivery, race or ethnicity, cigarette smoking, binge drinking, prepregnancy BMI, and periconceptional folic acid use
[e] Adjusted for maternal factors: age at delivery, race or ethnicity, level of education, cigarette smoking, binge drinking, and periconceptional folic acid use.
[f] Adjusted for maternal factors. age at delivery, race or ethnicity, prepregnancy BMI, and periconceptional folic acid use.
[g] Only male control infants included (n=2,364, 0.5% exposed).
[h] Adjusted for maternal factors. age at delivery, level of education, cigarette smoking, binge drinking, and prepregnancy BMI.
[i] Adjusted for maternal factors: age at delivery, cigarette smoking, binge drinking, prepregnancy BMI, and periconceptional folic acid use
[j] Adjusted for maternal factors: race or ethnicity, level of education, prepregnancy BMI, and periconceptional folic acid use

The National Birth Defects Prevention Study data offered several advantages in studying associations between periconceptional illicit drug use and birth defects. Because of the population-based and multistate ascertainment of case and control infants, the study population was geographically and racially diverse. Due to the large numbers, we were able to include relatively rare congenital malformations in

Table 8.4 Odds ratios and 95% confidence intervals (CIs) for the association between periconceptional stimulant use and selected birth defects. Data from the National Birth Defects Prevention Study, 1997-2003.

Defect	Total[a]	Stimulant exposed		Odds ratio (95% CI)	
		No.	%	Crude	Adjusted
None (controls)	4,704	27	0 6	Reference	Reference
Anencephaly, craniorachischisis	235	3	1 3	2.2 (0.7-7 4)	2 4 (0.7-8.1)[b]
Spina bifida	508	3	0 6	1 0 (0 3-3 4)	1 1 (0.3-3 7)[c]
Anotia, microtia	277	1	0 4	0.6 (0 1-4 6)	–
Dextrotransposition of the great arteries	329	2	0.6	1.1 (0 3-4 5)	–
Tetralogy of Fallot	468	1	0 2	0 4 (0 1-2 7)	–
Hypoplastic left heart syndrome	240	0	0 0	–	–
Coarctation of aorta	420	2	0.5	0.8 (0 2-3 5)	–
Pulmonary valve stenosis	559	1	0 2	0 4 (0 0-2 7)	–
Perimembranous VSD	898	5	0 6	1 0 (0 4-2 5)	1 1 (0 4-2 9)[d]
ASD secundum	917	5	0 5	1.0 (0.4-2 5)	0 8 (0 3-2.0)[e]
ASD not otherwise specified	276	2	0 7	1 3 (0 3-5 3)	–
Cleft lip ± cleft palate	1,217	9	0 7	1.3 (0 6-2 8)	1 0 (0 5-2 3)[f]
Cleft palate	656	4	0.6	1.1 (0 4-3 1)	1 2 (0.4-3 5)[g]
Esophageal atresia ± tracheoesophageal fistula	318	1	0 3	0 5 (0 1-4 0)	–
Anorectal atresia	458	3	0.7	1 1 (0 3-3 8)	1.1 (0.3-3.8)[h]
Hypospadias[c]	908	4	0 4	0 6 (0 2-1 7)	0 9 (0 3-2 8)[i]
Transverse limb deficiency	304	3	1 0	1.7 (0 5-5.7)	1.7 (0 5-5.9)[i]
Craniosynostosis	502	1	0 2	0 3 (0 0-2 6)	–
Diaphragmatic hernia	350	4	1 1	2.0 (0 7-5 8)	2 0 (0 7-5 8)[k]
Gastroschisis	432	9	2.1	3.7 (1.7-7 9)	1.0 (0.5-2.3)[l]

ASD, atrial septal defect, VSD, ventricular septal defect.

[a] Infants born to women with pre-existing diabetes type 1 or type 2 were excluded.

[b] Adjusted for maternal factors: age at delivery, race or ethnicity, level of education, binge drinking, prepregnancy body- mass index (BMI), and periconceptional folic acid use.

[c] Adjusted for maternal factors. age at delivery, race or ethnicity, cigarette smoking, binge drinking, and prepregnancy BMI.

[d] Adjusted for maternal factors: age at delivery, binge drinking, prepregnancy BMI, and periconceptional folic acid use

[e] Adjusted for maternal factors: age at delivery, race or ethnicity, level of education, cigarette smoking, and prepregnancy BMI.

[f] Adjusted for maternal factors: age at delivery, race or ethnicity, cigarette smoking, binge drinking, prepregnancy BMI, and periconceptional folic acid use.

[g] Adjusted for maternal factors level of education, binge drinking, prepregnancy BMI, and periconceptional folic acid use.

[h] Adjusted for maternal factors: age at delivery, level of education, binge drinking, prepregnancy BMI, and periconceptional folic acid use

[i] Only male control infants included (n=2,369, 0 8% exposed)

[i] Adjusted for maternal factors: age at delivery, cigarette smoking, binge drinking, and prepregnancy BMI.

[k] Adjusted for maternal factors· race or ethnicity, level of education, prepregnancy BMI, and periconceptional folic acid use.

[l] Adjusted for maternal factors age at delivery, race or ethnicity, level of education, cigarette smoking, binge drinking, prepregnancy BMI, and periconceptional folic acid use

this study. Many of the defects included have not been studied before in relation to periconceptional illicit substance use. Also, we implemented an extensive standardized interview that included detailed questions on illicit drug use and

important covariates. Every effort was made to conduct the postpartum interviews as close to the estimated date of delivery as possible; the average was 10 months after the estimated date of delivery with a range of 1.5-24 months. There was no difference in average time from the estimated date of delivery to the interview between exposed and unexposed subjects, not even after stratification for case/control status.

It is likely that the use of illicit drugs was underestimated in our study and other studies based on self-report. Respondents often falsely deny use because of the social stigma associated with use and fear of judgment or prosecution. Previous studies have shown that questionnaires identify 66%-82% of participants who test positive for drug use through toxicologic screening.[2,26,27] Misclassification of the exposure status of infants could attenuate the estimates toward the null value if it was non-differential between case mothers and control mothers, and it probably had a negative effect on the precision of our estimates. The ORs in this analysis would have been overestimated only if control mothers were more likely to deny illicit drug use than case mothers. Unintentional denial in the form of incomplete recall might also have been an issue in this study. However, among case-control studies of pregnancy outcome, few studies have reported evidence of recall bias.

In this study, we defined an infant as exposed if the mother reported illicit drug use in the period from one month before conception through the third month of pregnancy. The month before pregnancy was included because half of the pregnancies in the United States are unintended,[28] and such pregnancies are expected to be more prevalent among women who use illicit drugs.[29] It would also reduce social desirability bias due to women reporting abandonment of unhealthy behaviors in the first month of pregnancy. Because we collapsed the exposure data for the four-month period in which most congenital malformations originate, we did not know exactly at what time the women used the illicit drug. This could have led to underestimation or overestimation of our ORs because sporadic users might not have used a drug in the relevant exposure time window for the specific birth defect. Nevertheless, the associations between periconceptional cannabis and cocaine use and anencephaly and cleft palate, respectively, were found to be strongest in the etiologically relevant periods, indicating that the OR estimates for these associations in the entire periconceptional period were not overestimated.

Combinations of illicit substances can enhance the pharmacologic properties and physical effects of their components. Therefore, it can be hypothesized that certain combinations of illicit drugs could cause a specific congenital malformation. Because just a few women used cannabis and a second illicit substance, we were not able to

calculate adjusted ORs for multidrug cannabis users. However, there was no pattern of higher crude ORs among multidrug cannabis users compared with single-drug cannabis users. Furthermore, we did not find a pattern of defects for various combinations of substances among the infants exposed to three or more illicit drugs. Nevertheless, it is striking that among the 15 women who used illicit drugs from at least three different categories only two were control mothers.

Because we selected three exposures of interest and 20 outcomes, it is possible that the associations found were due to chance. The fact that the associations were strongest in the etiologically relevant periods, however, might indicate causality. Additionally, biologic explanations for these associations can be hypothesized. Δ^9-THC can bind and lead to inappropriate activation of the CB_1 and CB_2 receptor, the two cannabinoid receptors known to date.[19] In the early rat embryo, CB_1 receptor messenger RNA is expressed in some cells of the neural tube.[30] Because Δ^9-THC crosses the placenta,[31] the expression of CB_1 receptor messenger RNA suggests that exogenous cannabinoids might affect the developmental process of the neural tube, leading to neural tube defects. Prenatal marijuana exposure has been associated with neural tube defects in hamsters and rabbits.[32] Vasoconstriction and sudden hypertension caused by cocaine use may interrupt fetal blood supply[14,33] and could, therefore, result in an increased risk of cleft palate by decreasing the supply of essential nutrients to embryonic tissues.[34] We did not identify animal studies in which cocaine exposure was associated with cleft palate in particular.

Some alternative explanations could also be suggested for the associations found. Because anencephaly is diagnosed relatively early in pregnancy, women may choose an induced abortion, but cannabis users might get prenatal care too late for them to do so. However, we did not find a difference in the rate of induced abortions between exposed and unexposed anencephaly cases (41.7% versus 41.4%). Reverse causation bias can also be excluded because we found an increased risk for anencephaly only if cannabis was used in the relevant exposure period (the first month after conception). Furthermore, none of the exposed anencephaly cases was exposed to known teratogenic medications, excluding a confounding effect of medication use. One of the cocaine-exposed cleft palate cases was exposed to phenytoin and phenobarbital, anticonvulsants that have been associated with orofacial clefts.[35] If we exclude this case from the analyses, however, the adjusted OR is still increased (2.2, 1.0-4.9). Potential differential recall associated with time to interview might also explain the positive associations. On average, the mothers of unexposed anencephaly cases were interviewed sooner after the estimated date of delivery than cannabis-using anencephaly case mothers (10 vs. 13 months, p=0.10). However, it is unlikely that differential recall would be restricted to anencephaly cases only. For

cleft palate cases, we did not see differences in the average time to interview between cocaine-exposed and unexposed mothers.

The present findings showed very few positive associations between periconceptional illicit drug use and selected birth defects. Although the number of infants exposed to cocaine and stimulants was low, the statistical power of the data was sufficient to rule out two- to four-fold or greater increases in the risk of the selected birth defects. Cannabis use may be associated with an increased risk of anencephaly in offspring, and the risk of cleft palate appears to be increased for infants exposed to cocaine in the periconceptional period.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Part III

Methodological considerations

Chapter 9.1

Validation of maternal self-report in retrospective studies

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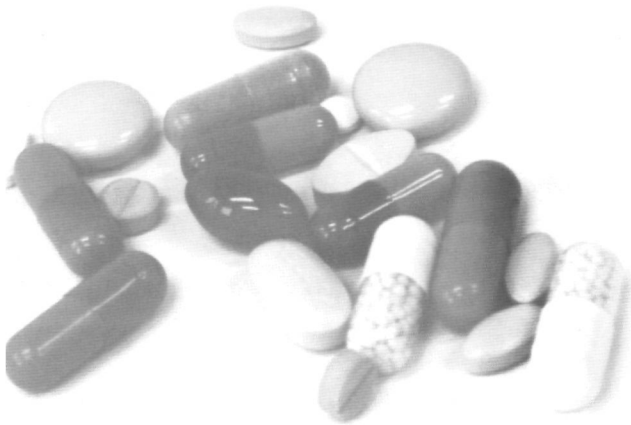
Abstract

Case-control studies are frequently performed in prenatal and perinatal epidemiology. For data collection, these studies often rely on self-administered questionnaires or personal interviews. Although of importance, validation of these measurement instruments received little attention in epidemiologic research so far. In this letter, we stress the need for more well-conducted validation studies in the field of prenatal and perinatal epidemiology. In addition, we comment on a validation study that was previously published in this journal.

In prenatal and perinatal epidemiology, case-control studies are frequently performed due to the low prevalence of the outcome parameter of interest, for example specific birth defects. As recorded data on various exposures during pregnancy and early life factors are usually not available, many researchers rely on self-administered questionnaires or personal interviews as a source of exposure information. However, the validity and reliability of the data collected by these methods depend on the ability of mothers to recall information accurately. Although it is generally recognized that validation of measurement instruments is essential in scientific research, it has received relatively little attention in epidemiologic research.

Most studies that validate maternal recall of pregnancy-related events compare the reported information with a 'gold standard' comparable with the methodology used in diagnostic research.[1] In these studies, results are reported in terms of sensitivity (the probability of being identified as exposed by the measurement instrument among those who were really exposed), specificity (the probability of being identified as unexposed among those who were really unexposed), positive predictive value (probability of really being exposed among those identified as exposed), and negative predictive value (probability of really being unexposed among those identified as unexposed). If a gold standard is not available, other outcome measures, such as the level of agreement and the kappa statistic,[2] can be used.

In an attempt to determine the reliability and accuracy of maternal recall for a number of pre- and perinatal factors, Rice *et al*.[3] compared information from maternal questionnaires with data from medical records. In addition to kappa coefficients, sensitivity and specificity of maternal reports were calculated with medical records as the gold standard. Unfortunately, incorrect formulas to calculate these measures were used in the statistical analysis. Sensitivity was calculated as the number of true positives / (the number of true positives + false positives) instead of the number of true positives / (the number of true positives + false negatives), and specificity as the number of true negatives / (the number of true negatives + false negatives) instead of the number of true negatives / (the number of true negatives + false positives). It also appears that the numbers of subjects for the two smoking variables were exchanged as the value of the kappa statistics could only be replicated using the figures presented for the opposite variable. One may expect the prevalence of smoking to be higher before rather than during pregnancy. Using the correct formulas and numbers, we recalculated the results reported by Rice *et al*. in Table 9.1.1. For some factors, including smoking during pregnancy and short labor, sensitivity and specificity changed considerably.

Table 9.1.1 Recalculated results for the agreement between maternal questionnaires and antenatal records.

	No. of true positives	No. of false positives	No. of false negatives	No. of true negatives	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Smoking pre-pregnancy[a]	12	5	3	77	0 80	0 94	0 71	0 96
Smoking during pregnancy[a]	7	1	2	84	0.78	0.99	0 88	0.98
Alcohol during pregnancy[b]	8	11	12	36	0 40	0 77	0.42	0 75
Short labor (<3 h)	3	8	4	68	0.43	0 90	0 27	0.94
Long labor (>36 h)[b]	1	7	0	74	1.00	0 91	0.13	1.00
Caesarean section	48	0	0	77	1 00	1 00	1.00	1.00
Emergency caesarean[b]	28	5	1	22	0.97	0 82	0 85	0.96
Use of forceps/ventouse	32	3	0	88	1.00	0 97	0.91	1.00
Special care baby unit	16	5	3	102	0 84	0 95	0.76	0 97
Admitted to hospital due to high blood pressure	12	8	0	101	1.00	0 93	0.60	1 00
Low birth weight baby (<2500 g)	18	3	2	101	0 90	0.97	0 86	0.98
Very low birth weight baby (<1500 g)	2	0	0	122	1.00	1 00	1 00	1.00

[a] As we have strong indications that the numbers of endorsed subjects for these variables were exchanged in the original report, numbers were corrected accordingly

[b] Using the original numbers of endorsed subjects on questionnaire and antenatal records, we were not able to reproduce the results. Therefore, the total numbers of endorsed subjects differ from those in the original report.

As the conclusions from the study by Rice *et al.* were based on the kappa statistics, which remained unchanged, their final conclusions still hold: mothers are able to provide accurate information on certain prenatal and perinatal characteristics, but not all. Because the validity and reliability of self-reported data are of major importance in prenatal and perinatal epidemiology, more well-conducted validation studies are necessary to assure the quality of these data.

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Chapter 9.2

Maternal recall of prescription medication use during pregnancy using a paper-and-pencil questionnaire: a validation study

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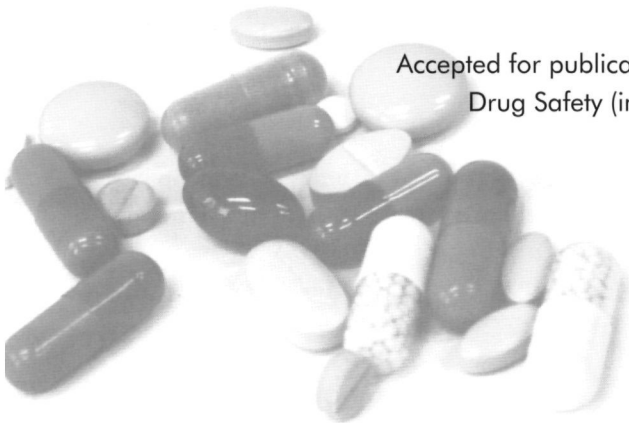
Abstract

Background In case-control studies that assess associations between medication use and birth defects, detailed information on type of medication and timing of use is essential to prevent misclassification. However, data on the accuracy of recall of medication use during pregnancy are scarce.

Objective To validate a self-administered questionnaire to assess prescription medication use in the three months before and during pregnancy.

Methods This validation study was imbedded in Eurocat Northern Netherlands, a population-based birth defects registry that covers 10% of all births in the Netherlands. The questionnaire was validated among 560 mothers of infants with major birth defects registered from January 1, 2009 through June 30, 2010 by comparing it to a reference standard consisting of pharmacy data which were checked for compliance by maternal interviews. Sensitivity and specificity were calculated to quantify validity for any prescription medication use, groups of medications, and individual medications. In addition, we determined whether maternal characteristics influenced disagreement between the questionnaire and the reference standard using logistic regression analyses.

Results The sensitivity for any prescription medication use was 0.57, ranging between 0.07 (dermatological corticosteroids) and 0.83 (antihypertensives) for medication groups and between 0.00 (naproxen) and 0.73 (salbutamol) for individual medications. Overall, specificity was high (0.93-1.00). Smoking during pregnancy and completing the questionnaire >2 years after delivery were associated with increased disagreement between the questionnaire for prescription medication use and the reference standard.

Conclusions The validity of the self-administered questionnaire for prescription medication use during pregnancy was moderate to poor for most medications and disagreement differed by some maternal characteristics. As in many epidemiologic studies similar questionnaires are being used to assess medication use, these studies may need additional data sources such as pharmacy records or prescription databases for medication use next to self-reported methods and take previous knowledge on the effect of questionnaire design into account.

Background

Prescription medication use is common during pregnancy with prevalence estimates ranging from 44 to 79% in several European countries.[1,3] Although it has firmly been established that some medications, including thalidomide and isotretinoin, are capable of producing birth defects, the human teratogenic risks are undetermined for 91% of pharmacological treatments approved for marketing in the United States since 1980.[4] This is due to a number of reasons. For ethical considerations, pregnant women are often excluded from pre-marketing clinical trials. In addition, results obtained from animal studies are not always predictive for a teratogenic effect in humans because of differences in factors such as anatomy, physiology, placentation, and embryonic development between laboratory animals and humans.[5] Prenatal medication exposure has also been associated with adverse long-term outcomes, including increased risks of childhood asthma[6] and attention deficit hyperactivity disorder,[7] but few studies have been conducted on this topic. Nevertheless, medication use is occasionally unavoidable in the treatment of women during pregnancy, for instance among women with epilepsy, diabetes, or severe hypertension. Therefore, epidemiologic studies that assess associations between medication use and birth defects and other developmental outcomes are needed.[8]

Since specific birth defects occur with a very low prevalence, studies on the teratogenicity of medication are mostly conducted using a case-control design, in which reliable data on medication intake are difficult to obtain.[9,10] In many studies in perinatal and pediatric epidemiology, prenatal medication exposure information was obtained through maternal self-report using questionnaires or interviews, but data on the validity of these methods are scarce. Previous validation studies indicate that the amount of data obtained depends on the type of medication of interest[9] and on the specificity of the questions asked with structured questions about indications and specific medication names being more successful in gathering reports of use than open-ended questions.[11] In general, however, mothers tend to underreport medication use during pregnancy.[9,12,13] In contrast, prescription and medical records or databases, which are commonly used as a reference standard (i.e. the measurement instrument that definitely determines whether the subject used medication or not), may overestimate prescription medication use as non-compliance may be particularly frequent among pregnant women.[14] As a result, previous studies may have underestimated the validity of self-reported modes of data collection. In addition, mothers may be more likely to recall medication use nowadays as many women use the Internet to retrieve pregnancy-related health information.[15] Over 50% of pregnant women who sought information online used the Internet to search for information about a treatment prescribed.[16] This may lead to an increased awareness of the potential teratogenic risks of medication use.

As detailed information on the type of medication and the timing of use is essential in case-control studies on the teratogenicity of prenatal medication exposure to prevent misclassification,[17] we validated a self-administered questionnaire to assess prescription medication use just before and during pregnancy. This questionnaire is part of a larger questionnaire being used in an ongoing study and similar sets of questions are incorporated into the questionnaires of many other large epidemiologic studies as well. In this study, which is by far the largest validation study conducted up to now, we compared this questionnaire to pharmacy data which were checked for compliance by maternal interviews. This is a better reference standard than those used in previous studies, as pharmacy records or databases alone, the commonly used reference standard, may overestimate prescription medication use. We also determined whether maternal and pregnancy characteristics influenced disagreement between questionnaire data on prescription medication use during pregnancy and the reference standard, an issue that has not been studied before.

Methods

Study population
This validation study was imbedded in Eurocat Northern Netherlands (Eurocat-NNL), a population-based birth defects registry which was established in 1981. The registry covers the provinces of Groningen, Friesland, and Drenthe with approximately 18,000 births annually (10% of all birth in the Netherlands). Infants and fetuses with major structural birth defects, monogenetic syndromes, and chromosomal anomalies are eligible for registration if the mother lived in the registry area at delivery. Infants and fetuses with only minor anomalies are excluded. As there is no lower age limit, induced abortions and miscarriages of fetuses with birth defects are included, but children with birth defects have to be notified to Eurocat-NNL before 16 years of age. Notification is voluntary and registry staff is actively involved in the search for eligible cases using multiple sources, including hospital registry databases, pathology reports, and cytogenetic reports. Parents have to give consent for registration, for which the positive response rate is 80%. Cases registered from January 1, 2009 through June 30, 2010 (*n*=1,105) were included in this validation study.

Ascertainment of prescription medication use
After consent for registration is received by Eurocat-NNL, an extensive questionnaire is sent to the parents. Through this questionnaire, information is collected on potential risk factors for birth defects, such as demographic factors, pregnancy and medical histories, lifestyle factors, including smoking, consumption of alcohol, and use of folic acid supplements, and occupational exposures, but not on medication use. For this validation study, an existing questionnaire on prescription medication

use during pregnancy was added to the regular Eurocat-NNL questionnaire. Following a general screening question about prescription medication use ("Did you use any medications in the three months before or during pregnancy that were prescribed to you by a medical doctor?", followed by an example of a prescription medication and a reference to look at the indication-oriented questions), women who responded positively were asked whether they used prescription medication for 11 specific indications/pharmacological groups and whether they used other prescription medications (Table 9.2.1). If medication use for a specific indication was reported, women were asked to specify the medication using an open-ended question and to give information on the timing of use (in the three months before pregnancy, gestational months 1-2, months 3-4, and/or months 5-9). The questionnaire on prescription medication use was developed using examples from previous studies that assessed prescription medication use through paper-and-pencil questionnaires. It was evaluated for content validity as part of a larger questionnaire on risk factors for birth defects among 15 mothers. A reminder was sent when the questionnaire was not returned within two months.

In addition to questionnaire data, information on prescription medication use was collected using the standard procedures implemented in Eurocat-NNL in 1997.[18] In the regular Eurocat-NNL questionnaire, consent is asked to obtain pharmacy records for the time period starting three months before pregnancy until delivery. In the Netherlands, almost everyone is registered with a single pharmacy and all pharmacies use computerized dispensing records. Therefore, medication records are virtually complete.[19] After the data on the medications dispensed in the requested period were received from the pharmacist, a telephone interview with the mother was conducted in which we asked whether she used the medications that were on the pharmacist's list and when and how often she used these. The majority of interviews took place more than one month after completion of the questionnaire. All medications that were actually used in the three months before pregnancy until delivery are registered in the Eurocat-NNL database as detailed as possible: name of medication, amount dispensed, daily dose, and time period of use.

All medications were coded using the Anatomical Therapeutic Chemical (ATC) classification system.[20] Only prescription medications were included as pharmacy records do not contain data on distribution of over-the-counter medication. In addition, use of anesthetics (ATC code N01), vaccinations (J05, J06, and J07), oral contraceptives (G03A), and folic acid supplements (B03BB01) were excluded from this study, because the first two are not dispensed by pharmacies and the latter may not be prescribed by a medical doctor or were not considered as medication by the

Table 9.2.1 Indication/pharmacological groups included in the questionnaire and their classification according to Anatomical Therapeutic Chemical (ATC) nomenclature.[20]

Indication/pharmacological group	Classification (ATC group)	Category[a]
Iron preparations	Iron preparations (B03A)	Pregnancy-related
Medication for nausea	Antiemetics (A03FA01, A04A, N05BA04, R06AD, R06AE)	Pregnancy-related
Sleep medication or sedatives	Hypnotics and sedatives (N05C)	Occasional/short-time use
Medication for anxiety or depression	Antidepressants, anxiolytics, and antipsychotics (N05A [excl. N05AB04], N05B, N06A)	Chronic use
Medication for asthma or chronic bronchitis	Anti-asthmatics (R03)	Chronic use
Medication for epilepsy	Anti-epileptics (N03A)	Chronic use
Medication for high blood pressure	Antihypertensives (C02, C07, C08, C09)	Chronic use
Medication for diabetes (including insulin)	Drug used in diabetes (A10)	Chronic use
Antibiotics	Antibiotics (D01, D06A, G01, J01, J02)	Occasional/short-time use
Prescribed pain medication	Anti-inflammatory/pain medication (M01, N02)	Chronic use
Prescribed anti-inflammatory medication	Anti-inflammatory/pain medication (M01, N02)	Chronic use
Other prescription medication		

[a] Mutually exclusive categories as reported by Bakker et al.[2]

respondent. Also, the Eurocat-NNL database is known to have incomplete data on these medications. We ordered the prescription medication used in the three months before or during pregnancy into three mutually exclusive categories as reported by Bakker et al.:[2] medication for chronic conditions, medication for occasional and short-time use, and pregnancy-related medication (Table 9.2.1). Medications for chronic conditions were not necessarily taken on a chronic basis but may have been used on an as needed basis only.

Statistical analysis

We defined prescription medication use according to the Eurocat-NNL database (pharmacy records in combination with maternal interviews) as our reference standard. To determine the validity of the self-administered questionnaire, sensitivity (proportion of women who reported prescription medication use among those who were really exposed) and specificity (proportion of women who did not report prescription medication use among those who were really unexposed) with 95% confidence intervals (CIs) were calculated (Table 9.2.2). These measures were calculated for any prescription medication use, for the three pre-defined medication categories, and for individual medications or groups of medications if there were at least 10 exposures according to the reference standard. As almost all birth defects originate in the first four months of pregnancy while other neonatal outcomes are probably more dependent upon late pregnancy exposure, we determined the validity for these two time periods separately as well. Because the screening question made it easy to skip the indication-specific questions, we performed a sensitivity analysis in which we excluded all women who falsely denied prescription medication use in the screening question. In addition, as some women reported in the questionnaire that they used, for instance, an antidepressant, but did not specify the name of the medication, we classified all women who reported only medication groups as being exposed to the most frequently used subgroup or individual medication in that group according to the reference standard.

Table 9.2.2 Calculation of measures to estimate validity in test research

	Reference standard	
	Positive (truly exposed)	Negative (truly unexposed)
Questionnaire positive	True positive (TP)	False-positive (FP)
Questionnaire negative	False-negative (FN)	True negative (TN)

Sensitivity = proportion of women who reported prescription medication use among those who were really exposed
= TP / (TP + FN)

Specificity = proportion of women who did not report prescription medication use among those who were really unexposed
= TN / (TN + FP)

Logistic regression analysis was used to evaluate whether selected maternal and pregnancy characteristics, including maternal age at delivery, level of education, gravidity, fertility problems prior to the index pregnancy, use of folic acid in the periconceptional period, smoking or alcohol consumption during pregnancy, place of birth, vital status at birth, type of birth defect, timing of diagnosis, and the time from delivery to completion of the questionnaire, influenced disagreement between the questionnaire data and the reference standard. These data were all available from the standard Eurocat-NNL questionnaire. The regression analyses were adjusted for smoking status during pregnancy and time from delivery to completion of the questionnaire whenever applicable, because these factors were associated with disagreement in the univariate analyses. All statistical analyses were performed using SPSS Version 16.0 for Windows (SPSS Inc., Chicago, IL).

Results

Of the 1,105 case mothers initially registered in Eurocat-NNL, 24 (2%) were ineligible because they lived outside the study area at delivery or their children were diagnosed with a birth defect ineligible for registration. In case of twin pregnancies in which both infants were affected with a birth defect ($n=3$), the mother was included in this validation study only once. A total of 777 completed questionnaires on prescription medication use were returned within the study period, yielding a response rate of 72%. Of these 777 cases, 13 mothers (2%) did not give permission to obtain pharmacy records and for 42 women (5%) pharmacy records were unavailable. On August 1, 2010, pharmacy records were not yet obtained and/or maternal interviews were not yet conducted for 162 cases (21%). Therefore, 560 women were included in this validation study. The median time between birth of the index child and completion of the questionnaire was 1.2 years (range: 0.1-15.3 years).

Prescription medication use was reported by 233 (42%) women in the questionnaire, whereas 389 (69%) women used prescription medication in the three months before and during pregnancy according to the reference standard. A total of 129 different individual medications or medication groups were reported in the questionnaire and 221 in the reference standard. In Table 9.2.3, the sensitivity and specificity are shown for the medication categories, medication groups, and selected individual medications. The sensitivity of the questionnaire for any prescription medication use was 0.57 (95% CI 0.52-0.62). After ordering the medications into the three pre-defined categories, the sensitivity decreased to 0.47 (95% CI 0.40-0.55) for medication for chronic conditions, with large numbers of false negatives for anti-inflammatory/pain medication and corticosteroids in dermatological preparations. Sensitivity was only 0.34 (95% CI 0.29-0.40) for medication for occasional and short-

Table 9.2.3 Validity comparisons of prescription medication use during the three months before and during pregnancy among mothers of infants with birth defects.

Medication group[a]	No. of subjects				Validity	
	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Any prescription medication	221	12	168	159	0 57 (0.52-0.62)	0.93 (0.89-0 97)
Medication for chronic conditions	84	11	94	371	0 47 (0.40-0.55)	0.97 (0 95-0 99)
Anti-asthmatics	23	3	6	528	0.79 (0.65-0 94)	0 99 (0 99-1.00)
Salbutamol	11	3	4	542	0 73 (0.51-0 96)	0 99 (0 99-1.00)
Antidepressants, anxiolytics, and antipsychotics	11	2	17	530	0 39 (0.21-0 57)	1 00 (0.99-1.00)
SSRIs	5	1	9	545	0 36 (0 11-0.61)	1 00 (0 99-1.00)
Antihypertensive medication	20	1	4	535	0 83 (0 68-0 98)	1 00 (0 99-1.00)
Methyldopa	3	0	7	550	0 30 (0 02-0 58)	1 00
Anti-inflammatory/pain medication	16	12	39	493	0 29 (0 17-0 41)	0 98 (0 96-0.99)
NSAIDs	8	8	32	512	0.20 (0 08-0 32)	0.98 (0.97-1.00)
Diclofenac	3	3	13	541	0.19 (0.00-0 38)	0 99 (0.99-1 00)
Ibuprofen	2	1	10	547	0 17 (0 00-0 38)	1 00 (0.99-1.00)
Naproxen	0	1	10	549	0 00	1 00 (0.99-1 00)
Antithrombotics	4	2	7	547	0.36 (0 08-0.65)	1.00 (0.99-1 00)
Corticosteroids, derm preparations	4	0	55	501	0 07 (0 00-0 13)	1.00
Medication for occasional and short-time use	95	8	183	274	0.34 (0 29-0 40)	0.97 (0.95-0 99)
Antibiotics, antifungals, and anti-infectives	76	7	143	334	0.35 (0.28-0 41)	0.98 (0.96-0 99)
Antifungals for dermatologic use	3	1	29	527	0.09 (0 00-0 19)	1.00 (0 99-1 00)
Gynecological anti-infectives	12	3	89	456	0.12 (0 06-0 18)	0.99 (0.99-1 00)
Antibacterials for systemic use	41	7	110	402	0.27 (0.20-0.34)	0.99 (0 97-1 00)
Amoxicillin	17	0	57	486	0.23 (0 13-0 33)	1.00
Amoxicillin and enzyme inhibitor	2	0	14	544	0 13 (0.00-0.29)	1.00
Doxycyclin	1	1	11	547	0.08 (0.00-0.24)	1 00 (0.99-1 00)
Nitrofurantoin	8	0	33	519	0.20 (0 07-0.32)	1 00
Trimethoprim	1	0	11	548	0.08 (0 00-0.24)	1.00
Ear, eye, nose, and throat preparations	12	1	54	493	0.18 (0 09-0.27)	1 00 (0 99-1 00)
Pregnancy-related medication	86	16	84	374	0 51 (0 43-0.58)	0 96 (0 94-0.98)
Antacids	6	3	17	534	0.26 (0.08-0.44)	0.99 (0 99-1.00)
Omeprazole	3	0	8	549	0.27 (0.01-0.54)	1 00
Antiemetics	23	4	14	519	0 62 (0.47-0 78)	0.99 (0 98-1.00)
Meclozine, combinations	7	0	13	540	0 35 (0.14-0.56)	1 00
Medication used in fertility treatment	10	2	28	520	0 26 (0.12-0.40)	1 00 (0 99-1 00)
Chorionic gonadotrophin	2	2	20	536	0 09 (0.00-0.21)	1 00 (0.99-1.00)
Clomiphene citrate	5	1	7	547	0 42 (0.14-0 70)	1 00 (0 99-1.00)
Follitropin alfa	1	1	11	547	0 08 (0.00-0 24)	1 00 (0 99-1.00)
Iron preparations	51	12	43	454	0 54 (0 44-0.64)	0 97 (0.96-0.99)
Ferrous fumarate	9	1	60	490	0.13 (0.05-0.21)	1 00 (0 99-1.00)
Ferrous sulphate	2	1	19	538	0 10 (0.00-0.22)	1 00 (0.99-1.00)

CI, confidence interval; FN, false-negative; FP, false-positive; NSAID, non-steroidal anti-inflammatory drug; SSRI, selective serotonin reuptake inhibitor; TN, true-negative; TP, true-positive.
[a] Only medication groups with at least 10 true exposures are shown.

time use, and 0.51 (95% CI 0.43-0.58) for pregnancy-related medication, among which antiemetics and iron preparations were reported relatively well with sensitivities of 0.62 (95% CI 0.47-0.78) and 0.54 (95% CI 0.44-0.64), respectively. For all medication groups, the sensitivity ranged between 0.07 (dermatological corticosteroids) and 0.83 (antihypertensive medication), while it ranged between 0.00

(naproxen) and 0.73 (salbutamol) for the individual medications that had at least 10 true exposures. Overall, specificity was high, ranging between 0.93 (any prescription medication) and 1.00 (30 individual medications or medication groups).

The sensitivity of the questionnaire for medication use in the first four months of pregnancy was comparable to or better than the sensitivity for medication use in the total period of three months before and during pregnancy (Table 9.2.4), except for any prescription medication use (0.49 vs. 0.57), medication for occasional and short-time use (0.29 vs. 0.34), antihypertensive medication (0.64 vs. 0.83), antibiotics, antifungals, and anti-infectives (0.30 vs. 0.35), antacids (0.10 vs. 0.26), and medication used in fertility treatment (0.04 vs. 0.26). For medication use in pregnancy months 5-9, the sensitivity of the questionnaire was generally lower than for medication use in early pregnancy, notably for any prescription medication use (0.42 vs. 0.49), pregnancy-related medication (0.35 vs. 0.45), anti-asthmatics (0.50 vs. 0.87), amoxicillin (0.16 vs. 0.24), ear, eye, nose, and throat preparations (0.11 vs. 0.23), and iron preparations (0.41 vs. 0.66). However, the sensitivity for antihypertensive medications was higher in pregnancy months 5-9 (0.88) when compared with pregnancy months 1-4 (0.64). The specificities for both time periods were high (0.93-1.00) and generally comparable to or better than those in the complete pregnancy period.

After excluding the false-negative reports for any prescription medication use (n=168), the sensitivity for almost all medication groups increased considerably (Table 9.2.5), but sensitivity remained below 0.50 for individual medications, with the exception of salbutamol (sensitivity 1.00). Only small or no decreases in specificity were observed. However, the large increase in sensitivity is partly biased as the number of false-negatives was artificially reduced in this sensitivity analysis, because some women who falsely denied prescription medication use in the screening question might also have denied use in the indication-specific questions if they had completed these. After reclassification of women who reported only medication groups instead of individual medications, the sensitivity of the questionnaire for selective serotonin reuptake inhibitors (0.36), methyldopa (0.30), amoxicillin (0.23), and ferrous fumarate (0.13) increased significantly to 0.50, 0.90, 0.46, and 0.54, respectively. However, application of this assumption increased the number of false positives, which slightly decreased specificity to 0.99 for selective serotonin reuptake inhibitors, 0.95 for amoxicillin, and 0.96 for ferrous fumarate.

Table 9.2.4 Validity comparisons of prescription medication use during pregnancy among mothers of infants with birth defects, stratified by pregnancy months.

Medication group	Pregnancy months 1-4			Pregnancy months 5-9		
	No. of truly exposed[a]	Sensitivity (95% CI)[b]	Specificity (95% CI)[b]	No. of truly exposed[a]	Sensitivity (95% CI)[b]	Specificity (95% CI)[b]
Any prescription medication	248	0.49 (0.43-0.55)	0 93 (0 90-0 96)	250	0.42 (0 36-0.49)	0.93 (0 90-0.96)
Medication for chronic conditions	91	0.51 (0.40-0 61)	0 96 (0 94-0.98)	77	0.51 (0.39-0.62)	0 97 (0 95-0.98)
Anti-asthmatics	15	0.87 (0.69-1.00)	0.99 (0.98-1.00)	16	0 50 (0 26-0 74)	0 99 (0 98-1.00)
Antidepressants, anxiolytics, and antipsychotics	17	0.41 (0.18-0.65)	1.00 (0 99-1.00)	9	–	–
SSRIs	11	0.36 (0.08-0.65)	1.00 (0.99-1.00)	4	–	–
Antihypertensive medication	11	0.64 (0 35-0 92)	1 00 (0 99-1.00)	16	0 88 (0 71-1 00)	1 00 (0 99-1.00)
Anti-inflammatory/pain medication	18	0 39 (0.16-0 61)	0.99 (0 98-1.00)	3	–	–
NSAIDs	12	0.25 (0.01-0 49)	0.99 (0 99-1.00)	1	–	–
Corticosteroids, dermatological preparations	25	0.08 (0.00-0 19)	1.00 (0.99-1.00)	24	0.04 (0.00-0 12)	1 00 (0.99-1.00)
Medication for occasional and short-time use	151	0.29 (0.22-0.36)	0.99 (0.97-1.00)	147	0 24 (0 18-0 31)	0 98 (0 97-0 99)
Antibiotics, antifungals, and anti-infectives	105	0.30 (0.22-0.39)	0.99 (0 98-1 00)	111	0 25 (0 17-0.33)	0 99 (0 98-1.00)
Antifungals for dermatological use	13	0.08 (0.00-0 22)	1 00	18	0.06 (0 00-0.16)	1.00
Gynecological anti-infectives	42	0.05 (0.00-0 11)	1.00 (0.99-1.00)	54	0 06 (0 00-0.12)	1 00
Antibacterials for systemic use	67	0.27 (0.16-0.37)	0.99 (0 98-1.00)	62	0 27 (0 16-0.39)	0 99 (0 98-1.00)
Amoxicillin	34	0.24 (0.09-0 38)	1.00	45	0.16 (0.05-0.26)	1.00 (0 99-1.00)
Nitrofurantoin	18	0.17 (0.00-0 34)	1 00	13	0 23 (0 00-0 46)	1 00
Ear, eye, nose, and throat preparations	31	0.23 (0.08-0.37)	1.00	27	0 11 (0 00-0.23)	1 00 (0 99-1 00)
Pregnancy-related medication	80	0.45 (0.34-0.56)	0.97 (0 96-0.99)	99	0 35 (0 26-0.45)	0 97 (0 96-0 99)
Antacids	10	0.10 (0.00-0 29)	1 00 (0.99-1.00)	9	–	–
Antiemetics	28	0.61 (0.43-0.79)	0.99 (0 99-1.00)	4	–	–
Meclozine, combinations	15	0 27 (0.04-0 49)	1.00	0	–	–
Medication used in fertility treatment	24	0.04 (0.00-0 12)	1 00	0	–	–
Chorionic gonadotrophin	10	0.00	1.00 (0 99-1.00)	0	–	–
Iron preparations	29	0 66 (0 48-0 83)	0.99 (0 98-1 00)	80	0 41 (0 30-0 52)	0 99 (0 97-1 00)
Ferrous fumarate	23	0 13 (0 00-0 27)	1 00 (0 99-1 00)	58	0 14 (0.05-0 23)	1 00 (0 99-1 00)
Ferrous sulphate	3	–	–	19	0.05 (0 00-0 15)	1 00 (0.99-1 00)

CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; SSRI, selective serotonin-reuptake inhibitor
[a] Truly exposed = true positives + false-negatives
[b] Sensitivity and specificity were only calculated for medication groups with at least 10 true exposures according to the reference standard

Table 9.2.5 Results of validity comparisons in the sensitivity analysis, excluding the false-negative reports on the screening question (n=168).

Medication group[a]	Validity	
	Sensitivity (95% CI)	Specificity (95% CI)
Medication for chronic conditions	0.71 (0.63-0 79)	0 96 (0.94-0 98)
Anti-asthmatics	0.92 (0.81-1 00)	0 99 (0.98-1 00)
Salbutamol	1 00	0.99 (0.98-1 00)
Antidepressants, anxiolytics, and antipsychotics	0 61 (0 39-0 84)	0 99 (0.99-1.00)
SSRIs	0.45 (0 16-0 75)	1.00 (0 99-1.00)
Antihypertensive medication	0.87 (0.73-1 00)	1.00 (0 99-1 00)
Methyldopa	0 30 (0.02-0 58)	1.00
Anti-inflammatory/pain medication	0 50 (0 33-0 67)	0.97 (0 95-0.99)
NSAIDs	0.36 (0.16-0.56)	0.98 (0 96-0.99)
Diclofenac	0 30 (0.02-0 58)	0.99 (0 98-1.00)
Corticosteroids, dermatological preparations	0 14 (0 01-0 26)	1.00
Medication for occasional and short-time use	0.59 (0.52-0.67)	0.97 (0.94-0 99)
Antibiotics, antifungals, and anti-infectives	0 59 (0 50-0.67)	0.97 (0.95-0.99)
Antifungals for dermatologic use	0 15 (0 00-0 31)	1 00 (0 99-1.00)
Gynecological anti-infectives	0 22 (0.11-0.33)	0.99 (0 98-1.00)
Antibacterials for systemic use	0 43 (0 33-0.53)	0.97 (0 94-0.99)
Amoxicillin	0 35 (0 22-0.49)	1.00
Amoxicillin and enzyme inhibitor	0 18 (0.00-0.41)	1.00
Nitrofurantoin	0 30 (0 12-0 47)	1 00
Ear, eye, nose, and throat preparations	0 35 (0 19-0 51)	1 00 (0 99-1.00)
Pregnancy-related medication	0.75 (0.67-0.83)	0.94 (0.91-0 97)
Antacids	0 38 (0 14-0.61)	0 99 (0.98-1 00)
Antiemetics	0 85 (0 72-0.99)	0 99 (0.98-1 00)
Meclozine, combinations	0.44 (0 19-0.68)	1.00
Medication used in fertility treatment	0 40 (0 21-0.59)	0 99 (0.99-1.00)
Chorionic gonadotrophin	0 13 (0 00-0.31)	0 99 (0.99-1 00)
Iron preparations	0 74 (0.64-0.84)	0 96 (0.94-0.98)
Ferrous fumarate	0 17 (0 07-0 28)	1 00 (0 99-1.00)
Ferrous sulphate	0 17 (0.00-0.38)	1 00 (0.99-1.00)

CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug; SSRI, selective serotonin reuptake inhibitor.
[a] Only medication groups with at least 10 true exposures are shown.

In Table 9.2.6 the adjusted odds ratios (ORs) and 95% CIs are shown for the associations between maternal characteristics and disagreement between questionnaire and the reference standard. Among younger women (<25 years of age), disagreement regarding medication for short-time use seemed to occur slightly more often than among women in the other age groups (adjusted OR 1.6, 95% CI 0.9-3.1). Having had fertility problems before the index pregnancy decreased maternal recall of pregnancy-related medication (adjusted OR 3.5, 95% CI 2.1-5.8), which was attributable to poor reporting of medication used in fertility treatment. Disagreement between questionnaire and the reference standard was also increased among women who smoked during pregnancy compared to non-smokers for any prescription medication (adjusted OR 1.7, 95% CI 1.1-2.6) and medication for chronic conditions (adjusted OR 1.8, 95% CI 1.1-2.8) and seemed to be increased

Table 9.2.6 Adjusted odds ratios and 95% confidence intervals for the association between disagreement of the reference standard and the questionnaire and selected maternal characteristics (*n*=560).

Characteristic	No.	%	Any prescription medication aOR[a] (95% CI)	Medication for chronic conditions aOR[a] (95% CI)	Medication for short-time use aOR[a] (95% CI)	Pregnancy-related medication aOR[a] (95% CI)
Maternal age at delivery						
<25 years	51	9 1	1.5 (0.8-2.9)	1 5 (0.7-3 3)	1 6 (0 9-3 1)	1.0 (0 4-2.4)
25-29 years	179	32.0	1 0 (0 7-1 6)	1 4 (0.8-2 3)	0 8 (0.5-1.2)	1.0 (0 6-1 8)
30-34 years	210	37.5	Reference	Reference	Reference	Reference
≥35 years	120	21.4	1 2 (0.8-2 0)	1 1 (0 6-2 0)	0 8 (0.5-1 3)	1.4 (0 8-2.5)
Low or intermediate level of education	333	59.5	1 0 (0 7-1 5)	1 1 (0.7-1 8)	1.1 (0 8-1 6)	1.2 (0.8-1.9)
First pregnancy	213	38.0	1 1 (0 8-1.6)	1.0 (0.7-1.6)	1.0 (0 7-1 5)	1.3 (0.8-2.0)
Fertility problems	87	15 5	1.2 (0.7-1.9)	0 7 (0 4-1 3)	1 1 (0.7-1 8)	3.5 (2 1-5.8)
Folic acid used						
Yes, in advised period	231	41.3	Reference	Reference	Reference	Reference
Yes, but not (completely) in advised period	235	42 0	0.8 (0.6-1 2)	1 0 (0.6-1 7)	1 1 (0.7-1 6)	0.7 (0.4-1 1)
No	90	16 1	1 1 (0 8-1.4)	1.2 (0.8-1.6)	1 1 (0 9-1 5)	1.1 (0 8-1 5)
Smoked during pregnancy	131	23 4	1.7 (1.1-2 6)	1.8 (1 1-2.8)	1 3 (0.9-2.0)	1.6 (1 0-2 6)
Alcohol consumption during pregnancy	99	17 7	1 0 (0.6-1.6)	0.8 (0 4-1.4)	0.6 (0.4-1 1)	0.7 (0.4-1.3)
Home birth	105	18 8	0 8 (0.5-1 3)	1.0 (0.6-1.7)	0.8 (0 5-1.2)	0 7 (0.4-1.2)
Vital status at birth						
Live-born	484	86 4	Reference	Reference	Reference	Reference
Miscarriage or stillbirth	15	2.7	1 7 (0.6-4 8)	2.4 (0 8-7 3)	2.6 (0.9-7.4)	0 4 (0 0-2.9)
Induced abortion	57	10 2	0.9 (0.6-1.2)	0 7 (0.5-1.2)	0 8 (0 6-1 1)	0 8 (0.5-1.2)
Chromosomal or monogenetic birth defect	148	26.4	0.9 (0.6-1 3)	1 4 (0.9-2 3)	0.9 (0 6-1 3)	0.8 (0.5-1 4)
Prenatal diagnosis of birth defect	131	23 4	1.2 (0 7-1.9)	1 0 (0.6-1 8)	0.8 (0.5-1.3)	0 8 (0.5-1 5)
Time between birth and completion questionnaire						
≤6 months	92	16.4	Reference	Reference	Reference	Reference
>6 months and ≤1 year	144	25.7	0 7 (0 4-1 3)	1 3 (0.6-2.6)	0 5 (0 3-0 8)	1 3 (0.6-2.8)
>1 year and ≤2 years	143	25.5	0 8 (0 5-1 5)	1.1 (0.6-2.3)	0 9 (0.6-1 6)	1.4 (0 6-3 0)
>2 years and ≤5 years	94	16.8	1 6 (0 9-2.9)	1.4 (0.7-3.0)	1 3 (0 7-2 3)	2.0 (0 9-4.6)
>5 years	98	15 5	1 9 (1 0-3 5)	1 9 (0 9-4.0)	1 2 (0 6-2 2)	3 3 (1 5-7 4)

aOR, adjusted odds ratio; CI, confidence interval

[a] Adjusted for smoking during pregnancy and time between birth and completion of the questionnaire. An increased OR denotes a higher level of disagreement between the reference standard and questionnaire data (i.e worse maternal recall) compared with the reference category

for medication for short-time use (adjusted OR 1.3, 95% CI 0.9-2.0) and pregnancy-related medication (adjusted OR 1.6, 95% CI 1.0-2.6) as well. Having had a miscarriage or stillbirth seemed to increase disagreement between questionnaire and the reference standard for medication for chronic conditions (adjusted OR 2.4, 95% CI 0.8-7.3) and for medication for short-time use (adjusted OR 2.6, 95% CI 0.9-7.4), but these results were based on small numbers. In addition, completing the questionnaire >2 years after delivery led to increased disagreement, in particular for any prescription medication and pregnancy-related medication. For this factor, the highest ORs were observed for completing the questionnaire >5 years after delivery compared to completing the questionnaire within six months after delivery for any prescription medication (adjusted OR 1.9, 95% CI 1.0-3.5), medication for chronic conditions (adjusted OR 1.9, 95% CI 0.9-4.0), and pregnancy-related medication (adjusted OR 3.3, 95% CI 1.5-7.4). The other maternal and pregnancy characteristics, including level of education, gravidity, use of folic acid, alcohol consumption during pregnancy, place of birth, type of birth defect, and timing of diagnosis, were not associated with disagreement between the questionnaire and the reference standard.

Discussion

In case-control studies in prenatal and perinatal epidemiology, self-completed questionnaires and personal interviews are often the only source of prenatal medication exposure information. Our results showed that the validity of a paper-and-pencil questionnaire to assess prescription medication use in the three months before and during pregnancy, which is being used in a similar format in many epidemiological studies, was moderate to poor for the majority of medications. Even for the etiologically relevant time period for birth defects (first four months of pregnancy) and for late pregnancy, women considerably underreported prescription medication use. Most maternal and pregnancy characteristics were not associated with disagreement between the questionnaire and the reference standard, except for having had fertility problems or a miscarriage or stillbirth related to the index pregnancy, smoking during pregnancy and completing the questionnaire >2 years after delivery, which were all associated with increased disagreement.

The major strength of this validation study is the use of pharmacy data which were checked for compliance by maternal interviews as reference standard. Compliance among pregnant women varies with the type of medication, with a high compliance for medication used in the treatment of chronic conditions (70-100%) and a low compliance for local or short-time treatment (12-77%).[14] Therefore, pharmacy data alone may overestimate prenatal medication exposure and, if used as a reference in

validation studies, may underestimate the validity of self-reported methods, especially for medication for occasional and short-time use. However, if women did not remember taking the medication or intentionally denied use during the interview, the reference standard could be prone to underreporting, which would have slightly inflated our sensitivity levels. Unfortunately, data on medication borrowing are not available for the Netherlands, but we may assume that this behavior is uncommon as almost all prescription medications are fully reimbursed in the Netherlands.[19] Although a home inventory would provide a superior measure of medication use,[21] this method of data collection cannot be used in retrospective study designs.

Other strengths of this study include the high consent rate (98%) to obtain pharmacy records, inclusion of pregnancies ending in a miscarriage or induced abortion, and availability of detailed data on demographic and other maternal and pregnancy characteristics. As a priori power analyses indicated that 400 women with complete information (i.e. data from the questionnaire and the reference standard) were sufficient to estimate sensitivity and specificity with satisfactory precision, we stopped data collection for this validation study on August 1, 2010. This yielded missing information on the reference standard for 162 women, who were therefore excluded from our analyses. As the women with missing data are a random sample of our complete study sample, these data may be regarded as 'missing completely at random' and handling the data in an available case approach thus gives unbiased results.[22]

However, our study also has some limitations. Rockenbauer et al.[23] showed that recall bias may be an important issue in case-control studies on the teratogenicity of medication use. However, other researchers did not find differences in reporting of exposure variables including medication use by case and control mothers.[24 25] Werler et al.[26] also found little evidence for recall bias, although three factors (use of birth control after conception, urinary tract or yeast infections, and a history of infertility) were reported more accurately by cases than by controls. We could not verify this because only infants with birth defects could be included in our study as Eurocat-NNL does not enroll healthy controls. However, questions about recall bias may be irrelevant as our study showed that the validity of the questionnaire is already poor among cases and therefore has very limited value in case-control studies of pregnancy outcome. Secondly, because pharmacy records do not contain information on over-the-counter medication and medication used during hospital stay, only the validity of the questionnaire for out-patient prescription medication use could be evaluated. Thirdly, the general screening question for prescription medication use as well as the relatively broad categories and open-ended questions used in our questionnaire may have resulted in a relatively high number of false-

negatives due to their inherent non-specificity However, such an approach is often still used in self-administered questionnaires Finally, validity could not be determined reliably for the majority of individual medications due to the low prevalence of use, despite the relatively large study population

Although maternal recall is reliable for pregnancy-related events such as severe obstetric complications,[27 28] mode of delivery,[28 29] birth weight,[28 30 31] and gestational age,[28 31 32] maternal recall of medical interventions was found to be poor in previous studies [33 34] The results of our study are consistent with validation studies conducted in the 1980s and 1990s that showed that the validity of self-reported data on prescription medication use during pregnancy is also low [9 12] As expected,[23 35] the recall sensitivity of medication used in the treatment of chronic conditions and pregnancy-related medication was higher than the recall sensitivity of medication used for short-time use, with the notable exceptions of psychiatric medication (sensitivity 0 39), anti-inflammatory and pain medication (sensitivity 0 29), antithrombotics (sensitivity 0 36), dermatological corticosteroids (sensitivity 0 07), antacids (sensitivity 0 26), and medication used in fertility treatment (sensitivity 0 26) Especially use of psychiatric medication may not only be poorly remembered, but may also be prone to social desirability bias Although included in the medication for chronic conditions, anti-inflammatory and pain medication as well as anxiolytics and antithrombotics were frequently used on an as needed basis instead of chronically, possibly leading to recall sensitivities similar to those for medication for short-time use The poor reporting of dermatological corticosteroids might result from the fact that we did not specifically ask for the use of dermatological preparations in the questionnaire, which was also true for medication used in fertility treatment Among the other pregnancy-related medication, antiemetics (sensitivity 0 62) and iron preparations (sensitivity 0 54) were relatively well reported, probably due to the impact of the pregnancy complication they were prescribed for However, these levels of sensitivity of maternal self-report still limit the use of this method of data collection for epidemiologic studies on pregnancy outcome

In accordance with previous studies,[27 33] we found that time from delivery until completion of the questionnaire influenced disagreement between the questionnaire and the reference standard considerably, especially concerning pregnancy-related medication To our knowledge, this is the first study that associated smoking during pregnancy with poorer maternal recall Some other studies found associations between recall sensitivity of pregnancy or birth characteristics and maternal age,[33] education,[32 36] and parity,[28 29 32 36] but others refuted these findings [26 30 33] Therefore, it is still uncertain which maternal and pregnancy characteristics influence recall of pregnancy events This issue should be the topic of future research as these

associations may introduce differential misclassification. To prevent recall bias, some researchers advocate the use of malformed infants or infants with a genetic disorder instead of infants without birth defects as a comparison group.[24,37,38] Indeed, in our study disagreement for prescription medication use was comparable for mothers of infants with chromosomal or monogenetic birth defects and mothers of infants with non-genetic birth defects, although mothers of infants with a genetic disorders appeared somewhat more likely to have disagreement for medication used for chronic conditions (adjusted OR 1.4, 95% CI 0.9-2.3). Also, women who had a miscarriage or stillbirth instead of a live-born infant with a birth defect seemed to have increased disagreement for all medication groups except for pregnancy-related medication. This should be taken into account in studies that include fetal deaths. As women with a miscarriage or stillbirth generally have a shorter pregnancy duration than women with a live-born infant, they are less likely to have used pregnancy-related medication and in particular iron preparations. Therefore, the probability of becoming a false-negative is lower for this small group of women, which may explain the decreased odds ratio for disagreement in this medication group.

In epidemiologic research, valid measurement of all study variables is essential to prevent information bias.[39] When non-differential in nature, misclassification of a dichotomous variable resulting from underreporting (i.e. low sensitivity), as observed in this validation study, usually biases effect estimates towards the null value. As a result, associations between the exposure, in this case prescription medication use during pregnancy, and the outcome may be obscured and the exposure may unjustly be regarded as safe. Differential misclassification may lead to either underestimation or overestimation of the true effect. Sensitivity analyses in which the potential effects of exposure misclassification are quantified may provide a solution when variables are measured imperfectly, although measuring exposures without error is definitely preferable.

Retrospective studies of pregnancy outcome need additional sources of data next to self-reported methods to validly gather information on prescription medication use during pregnancy. Pharmacy records or prescription databases may be a good data source provided that compliance is verified. If questionnaires or interviews are being used, open-ended questions should be avoided.[9,11,40,41] However, the sensitivity analysis in this validation study shows that indication-oriented questions lead to incomplete ascertainment of prenatal medication exposure as well, which limits their use in epidemiological studies. In fact, medication-specific questions, which include the names of the individual medications, should be used in self-reported modes of data collection to increase sensitivity. As there are thousands of medications, however, paper-and-pencil questionnaires and interviews cannot list all individual

medications that are being prescribed. Therefore, questions on medication use during pregnancy should be focused on medications of particular interest and/or on medications that are poorly reported, such as antidepressants, anti-inflammatory and pain medication, dermatological preparations, and specific antibiotics. Alternatively, a combination of indication-oriented and medication-specific questions may be used in computerized questionnaires in which only relevant medications will be visible to the respondent. Whatever method of data collection is being used, interviews and questionnaires should be completed before or as shortly after delivery as possible to ensure better recall of prescription medication use during pregnancy.

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Chapter 10

Assessing the effect of exposure misclassification on cannabis-birth defect associations: an application of Monte Carlo simulations and Bayesian models

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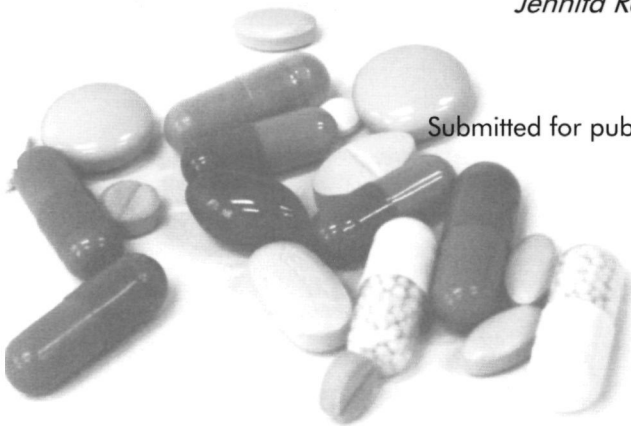
Abstract

Background

Studies on associations between periconceptional cannabis exposure and birth defects have mainly relied on self-reported exposure. Therefore, results may be biased due to exposure misclassification. The aim of this study was to quantify the potential effects of exposure misclassification.

Methods

We estimated odds ratios (ORs), adjusted for confounding and exposure misclassification, for associations between periconceptional cannabis use and seven birth defects using data from the National Birth Defects Prevention Study from 1997-2005. Monte Carlo simulation techniques were used to assess effects of misclassification with assumed sensitivity of reported cannabis use set at 0.80, 0.65, and 0.50. Additionally, four Bayesian models were implemented based on various assumptions concerning the sensitivity of self-reported cannabis use.

Results

The unadjusted results showed an association between cannabis use and anencephaly (OR 2.2, 95% confidence interval 1.1-3.2) which remained after adjustment for potential exposure misclassification. Initially, no associations were observed between cannabis use and the other birth defect categories assessed, but after adjustment for misclassification, cannabis use was associated with esophageal atresia (posterior OR 1.7, 95% credible interval (CRI) 1.0-2.9), diaphragmatic hernia (posterior OR 1.8, 95% CRI 1.1-3.0), and gastroschisis (posterior OR 1.7, 95% CRI 1.2-2.3).

Conclusions

Exposure misclassification may have obscured some cannabis-birth defect associations. Although theoretically a Bayesian approach is preferable, in practice we observed few differences in the OR estimates between the Monte Carlo simulations and the Bayesian approach.

Background

Valid measurement of exposures, outcomes, and potential confounders is essential in epidemiologic research to prevent information bias [1] In case-control studies, non-differential or differential misclassification may be present when exposure information is collected after the outcome has occurred Non-differential misclassification of a dichotomous variable usually biases results towards the null value [2,3] Differential misclassification, on the other hand, may lead to either underestimation or overestimation of the true effect [1] Multiple methods to correct for potential biases due to misclassification in observational studies have recently been published [4-7] Although misclassification frequently occurs in epidemiologic research, methods to quantify the resulting bias are rarely used because of complexity of reporting and lack of appropriate software [4,8] However, these issues do not justify lack of attention to potential bias in observed exposure-outcome associations that may result from ignoring misclassification [9,10] In this paper, we present a case study in which adjustments for exposure misclassification were made by means of relatively easy to use Monte Carlo simulations and more sophisticated Bayesian methods

According to the National Survey on Drug Use and Health 2008-2009, 7 7% of U S women aged 15-44 years reported use of cannabis in the past month [11] Although maternal cannabis use generally does not appear to be associated with the occurrence of major birth defects,[12-14] increased risks of gastroschisis,[15] isolated simple ventricular septal defects,[16] and anencephaly[14] have been reported after prenatal cannabis exposure As most of these studies used a case-control design and all used self-reported modes of data collection for exposure assessment (either maternal interviews or medical chart reviews), non-differential or differential misclassification of cannabis use may have occurred and subsequently may have biased the results It is very likely that the use of cannabis was underestimated in these studies, because some subjects would have falsely denied use for fear of judgment or prosecution [17] Although misclassification of cannabis use during pregnancy was generally acknowledged, no attempts were made to quantify the effect of exposure misclassification on cannabis-birth defect associations In this study, we used data from the National Birth Defects Prevention Study to estimate odds ratios and interval estimates adjusted for exposure misclassification for the associations between periconceptional cannabis use and selected birth defects

Methods

Data

The National Birth Defects Prevention Study is an ongoing multi-site population-based case-control study of more than 30 types of major birth defects that started enrollment of women with an estimated date of delivery on or after October 1, 1997.[18] Case infants (live-born, stillborn, or induced abortions) were identified using existing birth defects surveillance systems in Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah. Information on birth defects abstracted from hospital records was reviewed by a clinical geneticist at each study center to determine eligibility. The methods used for case classification in the NBDPS have been described in detail elsewhere.[19] Control infants were randomly selected from all live-born infants without any major birth defect from the same geographical area and time period using either hospital birth records (Arkansas, California, Georgia 1997-2000, New York, and Texas) or birth certificates (Georgia 2001-2005, Iowa, Massachusetts, New Jersey, North Carolina, and Utah). Computer-assisted telephone interviews were conducted with the mothers of case and control infants between 6 weeks and 24 months after the estimated date of delivery, including questions on demographic factors, medical and pregnancy history, lifestyle, and occupation. For the time period of interest, the interview participation rate was 69% for case mothers and 66% for control mothers.

In this study, exposure to cannabis was defined as any reported use of marijuana or hashish in the period from one month before pregnancy to the end of the third month of pregnancy (periconceptional period). Only case and control infants whose mothers did not report use of any illicit drug in the three months before pregnancy and during the entire index pregnancy were considered unexposed.

The current study base included all case and control infants born from October 1, 1997 through December 31, 2005, whose mothers completed the interview (*n*=18,745 and 6,703, respectively). This dataset overlaps to a large extent with the dataset used by van Gelder *et al.* to study associations between periconceptional illicit drug use and birth defects,[14] but it contains two additional years of data. Only case infants diagnosed with one of the 20 birth defect categories selected by van Gelder *et al.*[14] (*n*=14,429) were included in this study. Infants born to women who reported pre-existing diabetes type 1 or type 2 (298 cases and 39 controls) were excluded because of the strong association with major birth defects,[20] as well as infants only exposed to other types of illicit drugs or with missing information on illicit drug exposure because of our exposure definition (272 cases and 108 controls). Eventually, we analyzed data on 13,859 case infants and 6,556 control infants.

Statistical analysis

We first replicated our earlier analyses[14] using the updated dataset. In these analyses, multivariable logistic regression techniques were used to calculate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for periconceptional cannabis use and each of the selected birth defects. The same confounder set used in the previous study, consisting of maternal age at delivery (continuous), race/ethnicity (non-Hispanic white, other), level of education (≤12 years, >12 years), smoking in the periconceptional period (yes/no), binge drinking in the periconceptional period (≥4 drinks per episode, yes/no), prepregnancy body mass index (BMI, continuous), and any use of folic acid or multivitamins in the month before pregnancy or in the first month of pregnancy (yes/no), was included in all current multivariable analyses. The replication analyses were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL).

As data on the validity of the interviews for periconceptional cannabis use were not available, we used information on sensitivity of self-reports from the literature. We are not aware of any studies that provide information on accuracy of cannabis reporting among mothers of infants with birth defects. However, we identified five studies which determined the sensitivity of interview data on cannabis use among pregnant and postpartum women (Table 10.1).[21-25] These studies reported sensitivities ranging from 0.58 to 0.82. Because falsely reporting cannabis use is very unlikely, we assumed specificity to be 1.00 in all analyses. These parameters were used in Monte Carlo simulations and Bayesian methods to adjust the cannabis-birth defect associations for non-differential or differential misclassification. These analyses were performed using R version 2.12.2 for Windows[26] for seven birth defect groups, which were selected based on the results of the replication analyses: all defects which seemed to have an elevated (anencephaly, esophageal atresia, diaphragmatic hernia, and gastroschisis) or decreased (hypospadias) OR for periconceptional cannabis use, one defect that has been associated with periconceptional cannabis

Table 10.1 Studies that reported sensitivity values for interview data on cannabis use during pregnancy.

Authors	Source population	Years of data collection	Reference	Sensitivity (No. true positives / total No. positives)
Frank et al [22]	Boston, USA	1984-1986	Urine samples	0 76 (94/123)
Hingson et al [21]	Boston, USA	1984	Urine samples	0.82 (23/28)
Jacobson et al.[24]	Detroit, USA	NR	Antenatal interview	0 75 (44/59)
Ostrea et al.[25]	Detroit, USA	NR	Maternal hair + meconium	0 58
Zuckerman et al [23]	Boston, USA	1984-1987	Urine samples	0 74 (149/202)

NR, not reported.

use in previous studies, but not in our earlier study (perimembranous ventricular septal defects), and one defect with a relatively large case group which was not associated with periconceptional cannabis use (cleft lip ± cleft palate).

Monte Carlo simulations

In the Monte Carlo simulations, we specified three fixed values for the probability that a mother who used cannabis in the periconceptional period accurately reported that in the interview. The assumed values for these sensitivities (i.e. proportion of women who reported cannabis use among those that were really exposed) were 0.80, 0.65, and 0.50. The replication analyses served as the reference scenario (Scenario 1). In the analyses in which non-differential misclassification of periconceptional cannabis exposure was simulated (Scenario 2), the value for recall sensitivity among cases and controls was equal by definition. For the simulations regarding differential misclassification, sensitivity was assumed to be 0.05 or 0.10 lower among controls than among cases, reflecting recall bias (Scenario 3). Under our assumption that the specificity of reported cannabis use was 1.0, the probability of being exposed while classified as unexposed (*probD*) for the seven separate birth defect groups and the control group is:

$$probD = ((1 - sensitivity) / sensitivity) \times (E_1 / E_0)$$

where E_1 is the observed number of exposed subjects and E_0 is the observed number of unexposed subjects within the outcome group selected. Subsequently, these probabilities were applied to the individual records in the dataset to allocate a corrected cannabis exposure status to subjects categorized as non-exposed by the interview. Within this reconstructed dataset, the regression coefficients for the associations between periconceptional cannabis use and the selected birth defects adjusted for the same covariates as in the replication analyses were estimated using multivariable logistic regression analysis.

For each scenario, this process was repeated 10,000 times to create a distribution of regression coefficients adjusted for exposure misclassification, of which the mean coefficient was exponentiated to obtain the OR of interest. As the conventional 95% CI calculated from the mean standard error (SE) of the regression coefficients would account for sampling error only,[27] some adjustments had to be implemented. Greenland[28] suggests a multiple equations procedure, but given the fact that we had a rather simplified problem in this case study as we only assumed exposure misclassification, we used a single equation procedure based on the law of total variance to calculate the total SE:

$$SE_{total} = \sqrt{(\text{mean}(SE^2) + \textit{variance of the coefficients across Monte Carlo simulations})}$$

In a random sample of simulations, this equation yielded similar standard errors compared with the methods described by Greenland (data not shown).[28] The R script used for the Monte Carlo simulations is shown in Appendix 10.1.

Bayesian methods

Following the framework of MacLehose *et al.*,[6] we conducted Bayesian uncertainty analyses conditional on prior hypotheses generated from published studies. In short, three models were jointly estimated: an outcome model, an exposure model, and a measurement model. In the outcome model, the odds of an infant having the selected birth defect was modeled with a logistic regression model conditional on the (unknown) periconceptional cannabis exposure status and the potential confounders assuming no interaction between cannabis use and other factors. We used a non-informative normal distribution with mean=0 and variance=10^6 for the prior distribution of the intercept. Informative priors, for which prior studies and expert opinions were used, were placed on the remaining parameters (Appendix 10.2). As an example, we assumed folic acid supplementation to have no additional protective effect on folate-sensitive birth defects (normal distribution, mean=0, variance=0.13), because all pregnant women in our study were exposed to relatively high levels of folic acid through food fortification.[29] Priors on coefficients for which no information from previous studies was available and the prior on the effect of periconceptional cannabis use on the risk of birth defects were kept relatively vague (normal distribution, mean=0, variance=0.67) as we were uncertain about the magnitude of the ORs for these associations.

In the exposure model, we modeled the probability of true exposure to cannabis in the periconceptional period conditional on a set of predictors, which consisted of all potential confounders and paternal cannabis use, which is highly predictive of maternal illicit drug use.[30,31] However, as is discussed by MacLehose *et al.*,[6] it is difficult to inform priors for parameter estimates because the outcome of interest, true exposure to cannabis, is generally not observed in studies. Therefore, we also placed vague priors (normal distribution, mean=0, variance=0.67) on the coefficients in this model.

Finally, in the measurement model we modeled the probability of reporting periconceptional cannabis use during the interview dependent on the true (but unobserved) exposure status and the case/control status, which allowed us to introduce differential misclassification. Because we assumed specificity to be 1.00, we could simplify the measurement model used by MacLehose *et al.*[6] to:

$$PR(can_i^{int} = 1) = \alpha_0 \times can_i^{true} \times (1 - BD_i) + \alpha_1 \times can_i^{true} \times BD_i$$

where can_i^{int} is the periconceptional cannabis exposure status the woman reported in the interview, α_0 is the sensitivity of reported cannabis use among control mothers, can_i^{true} is the unobserved true periconceptional cannabis exposure status, BD_i is case/control status, and α_1 is the sensitivity of reported cannabis use among case mothers. The exposure and measurement models were used to impute values of can_i^{true} in a way similarly to that used with Bayesian missing data techniques.[6] These imputed values were then used to estimate the associations between periconceptional cannabis use and the selected birth defects.

To quantify the potential effects of exposure misclassification on the cannabis-birth defect associations observed, we implemented four scenarios that specified α_0 and α_1 in the measurement model. In Scenario 1, the reference scenario, we assumed sensitivity to equal 1.00, which resulted in a standard Bayesian logistic regression model. Scenario 2 is based on the assumption that cannabis exposure status was non-differentially misclassified with the sensitivity of reported use fixed at the same levels as in the Monte Carlo simulations. For Scenario 3, it was assumed that the cannabis exposure status was differentially misclassified with the same amounts as in the Monte Carlo simulations. Scenario 4 was based on the assumption that the sensitivities used in the measurement model are not exactly known. For the prior distribution of the sensitivity, a beta distribution was chosen with prior parameters selected to reflect a priori beliefs concerning reported cannabis use, i.e. sensitivity with a mean of 0.7 (ba_1) and a standard deviation of 0.1 (ba_2).

We conducted a sensitivity analysis to determine the influence of our prior assumptions in the exposure and outcome models by placing vague priors on all coefficients in every model. All models were fitted using Markov chain Monte Carlo algorithms, which were run for 20,000 iterations with the first 1,000 iterations excluded as a burn-in period. After the burn-in period, the iterations of the algorithm were random draws from the posterior distributions of interest, of which the median was exponentiated to obtain the OR of interest. We exponentiated the 2.5th and 97.5th percentile of the random draws to obtain 95% posterior credible intervals. The R script and model specifications used are shown in Appendix 10.3.

Results

A total of 825 mothers (4.0%) reported use of cannabis in the periconceptional period: 4.1% of the case mothers and 3.8% of the control mothers. The ORs for periconceptional cannabis use adjusted for confounding observed in the original and updated datasets for each of the birth defects studied are shown in Table 10.2. In general, the results from the replication analyses were comparable with the ORs reported earlier.[14] In the updated dataset, periconceptional cannabis use was more strongly associated with anencephaly (adjusted OR 2.2, 95% CI 1.3-3.7) and indications were found for associations with esophageal atresia (adjusted OR 1.4, 95% CI 0.8-2.4) and diaphragmatic hernia (adjusted OR 1.4, 95% CI 0.9-2.2). No associations were observed between cannabis use in the periconceptional period and any of the other 17 birth defect categories.

Table 10.2 Observed adjusted odds ratios and 95% confidence intervals for the associations between periconceptional cannabis use and selected birth defects Data from the National Birth Defects Prevention Study, 1997-2005

Birth defect	NBDPS 1997-2003[a]		NBDPS 1997-2005	
	No. of exposed cases / total no. of cases	Adjusted OR (95% CI)[b]	No. of exposed cases / total no. of cases	Adjusted OR (95% CI)[b]
None (controls)	189 / 4,866	Reference	251 / 6,556	Reference
Anencephaly/craniorachischisis	12 / 244	1 7 (0 9-3.4)	18 / 329	2 2 (1 3-3 7)
Spina bifida	20 / 525	1.0 (0 6-1.6)	24 / 703	0 9 (0 6-1.4)
Anotia, microtia	11 / 287	1 0 (0 5-2.0)	13 / 394	0 9 (0 5-1.7)
D-transposition great vessels	9 / 336	0 7 (0 3-1.4)	14 / 451	0 8 (0 5-1.5)
Tetralogy of Fallot	19 / 486	1 1 (0 6-1.8)	24 / 657	1.1 (0 7-1.7)
Hypoplastic left heart	7 / 247	0.7 (0.3-1.6)	10 / 355	0 8 (0 4-1 5)
Coarctation of the aorta	15 / 433	1 0 (0.6-1.8)	21 / 618	1 2 (0 7-1 9)
Pulmonary valve stenosis	24 / 582	1 2 (0 8-1.9)	32 / 850	1 0 (0 7-1.5)
Perimembranous VSD	34 / 927	0 9 (0 6-1.4)	52 / 1363	1 0 (0 8-1 4)
ASD secundum	31 / 943	0 7 (0 5-1.0)	54 / 1,465	0 8 (0 6-1 1)
ASD not otherwise specified	14 / 288	1 2 (0 7-2.2)	22 / 500	1 1 (0 7-1 8)
Cleft lip ± cleft palate	61 / 1,269	1 0 (0 7-1.4)	82 / 1,735	1 0 (0 8-1.3)
Cleft palate	25 / 677	0 8 (0 5-1.3)	38 / 907	1 0 (0 7-1 5)
Esophageal atresia	12 / 329	1 2 (0 6-2.2)	17 / 419	1 4 (0 8-2 4)
Anorectal atresia	13 / 468	0 7 (0.4-1.2)	19 / 605	0.8 (0 5-1 3)
Hypospadias[c]	20 / 924	0 7 (0 4-1.2)	32 / 1,291	0.8 (0 5-1 2)
Transverse limb deficiency	14 / 315	1.1 (0 6-2.0)	16 / 404	1.0 (0 6-1 7)
Craniosynostosis	16 / 517	1 0 (0.5-1.7)	21 / 786	0.8 (0 5-1 3)
Diaphragmatic hernia	19 / 365	1 3 (0 8-2.2)	25 / 498	1.4 (0 9-2 2)
Gastroschisis	62 / 485	1.3 (0.9-1.8)	82 / 688	1 2 (0 9-1 7)

ASD, atrial septal defect; CI, confidence interval, NBDPS, National Birth Defects Prevention Study, OR, odds ratio, VSD, ventricular septal defect.
[a] As reported by van Gelder et al[14]
[b] Adjusted for maternal factors: age at delivery, race or ethnicity, level of education, cigarette smoking, binge drinking, prepregnancy BMI, and periconceptional folic acid use.
[c] Only male control infants included (1997-2003: n=2,452, 4 1% exposed, 1997-2005 n=3,316, 4 1% exposed)

The crude ORs and 95% uncertainty intervals (UIs) for the associations between periconceptional cannabis use and the seven selected birth defects after adjustment for non-differential misclassification (Scenario 2) in the Monte Carlo simulations are shown in Table 10.3. These adjustments did not substantially change the crude OR estimates assuming no misclassification (Scenario 1), with the exception of the ORs for gastroschisis, which clearly shifted further away from the null with decreasing levels of sensitivity. After correction for confounding, we observed associations between periconceptional cannabis use and anencephaly with adjusted ORs of 2.0 (95% UI 1.2-3.3), 1.8 (95% UI 1.1-3.1), and 1.7 (95% UI 1.1-1.8) for assumed sensitivities of 0.80, 0.65, and 0.50, respectively (Figure 10.1A, Scenario 2, squares). In addition, cannabis use in the periconceptional period appeared to be associated with diaphragmatic hernia (adjusted OR 1.4, 95% UI 0.9-2.0 for all levels of sensitivity) and gastroschisis (sensitivity 0.80: adjusted OR 1.5, 95% UI 1.1-2.1; sensitivity 0.65: adjusted OR 1.8, 95% UI 1.3-2.4]; sensitivity 0.50: adjusted OR 2.2, 95% UI 1.6-2.9) after adjustment for non-differential misclassification and confounding.

As expected, the elevated ORs for these three defects diminished after adjusting for the possibility of recall bias (Scenario 3), but still indicated increased risks of anencephaly and gastroschisis after periconceptional cannabis use. For hypospadias, adjustments for both non-differential and differential misclassification reduced the ORs with the lowest value being 0.6 (95% UI 0.4-0.8; Scenario 3, sensitivity$_{cases}$ 0.65, sensitivity$_{controls}$ 0.55).

In the Bayesian assessment, the observed ORs for the associations between the potential confounders and the selected birth defects (Appendix 10.4) were for the most part in line with the prior specifications (Appendix 10.2). The posterior ORs and 95% credible intervals (CRIs) for the associations between periconceptional cannabis use and the seven selected birth defects are shown in Figure 10.1. For anencephaly, perimembranous ventricular septal defects, and cleft lip ± cleft palate, the Bayesian approach yielded results comparable to those of the Monte Carlo simulations for Scenarios 1-3. For esophageal atresia and diaphragmatic hernia, the Bayesian approach seemed to result in OR estimates further away from the null than the Monte Carlo simulations after adjustment for either non-differential or differential exposure misclassification with ORs up to 1.6 (95% CRI 0.9-2.5) and 1.8 (95% CRI 1.2-2.7) for esophageal atresia and diaphragmatic hernia, respectively (Scenario 2, sensitivity 0.50). Estimates for Scenario 2 and 3 from the Bayesian approach were shifted toward the null compared with the Monte Carlo simulations for the association between periconceptional cannabis use and hypospadias. For gastroschisis, the

Table 10.3 Crude odds ratios and 95% uncertainty intervals for the associations between periconceptional cannabis use and selected birth defects adjusted for non-differential exposure misclassification using Monte Carlo simulations. Data from the National Birth Defects Prevention Study, 1997-2005.

Birth defect	No misclassification	Sensitivity 0.80	Sensitivity 0.65	Sensitivity 0.50
	Crude OR (95% CI)	Crude OR (95% UI)	Crude OR (95% UI)	Crude OR (95% UI)
Anencephaly/craniorachischisis	1.45 (0 89-2 38)	1 45 (0 89-2.37)	1.46 (0 91-2 34)	1.47 (0 94-2.28)
Perimembranous VSD	1.00 (0 74-1 35)	0 99 (0 74-1.34)	1.00 (0 74-1 33)	1.00 (0.76-1 31)
Cleft lip ± cleft palate	1.23 (0.95-1 59)	1 23 (0 96-1.59)	1.24 (0.97-1.58)	1.24 (0.99-1.56)
Esophageal atresia	1 06 (0 64-1.75)	1 06 (0 64-1 74)	1.06 (0 65-1.71)	1 06 (0 68-1 66)
Hypospadias	0 59 (0 40-0 88)	0.59 (0.40-0 87)	0.59 (0.41-0.85)	0.58 (0 41-0 82)
Diaphragmatic hernia	1 33 (0 87-2 02)	1.33 (0.87-2.02)	1 33 (0 89-2 00)	1 34 (0 92-1 96)
Gastroschisis	3.40 (2.61-4.42)	3 48 (2 68-4 53)	3.59 (2 77-4 64)	3 77 (2.95-4.82)

CI, confidence interval; OR, odds ratio; UI, uncertainty interval, VSD, ventricular septal defect

Bayesian approach yielded lower OR estimates for Scenario 2 than the Monte Carlo simulations, but these still indicated an increased risk of this defect after exposure to cannabis in the periconceptional period (sensitivity 0.80: OR 1.5, 95% CRI 1.1-2.1; sensitivity 0.65: OR 1.6, 95% CRI 1.2-2.2; sensitivity 0.50: OR 1.6, 95% CRI 1.2-2.2). Estimates from Scenario 4, which treated the sensitivity as unknown, showed
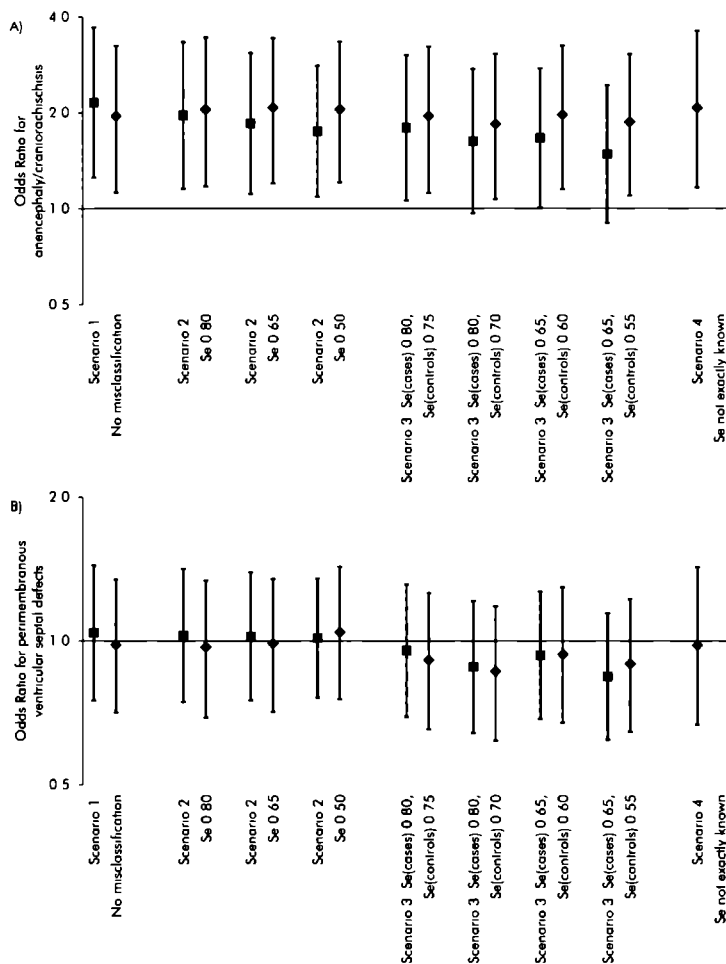
(Figure 10.1 continues)

Figure 10.1 Confounder-adjusted and posterior odds ratios with 95% uncertainty/credible intervals for the association between periconceptional cannabis use and A) anencephaly/craniorachischisis, B) perimembranous ventricular septal defects, C) cleft lip ± cleft palate, D) esophageal atresia, E) hypospadias, F) diaphragmatic hernia, and G) gastroschisis, adjusted for exposure misclassification using Monte Carlo simulations (squares) and Bayesian methods (rhombi). Data from the National Birth Defects Prevention Study, 1997-2005. Se, sensitivity.

(Figure 10.1 continues)

C)

Odds Ratio for
cleft lip ± cleft palate

E)

Odds Ratio for
hypospadias

D)

Odds Ratio for
esophageal atresia
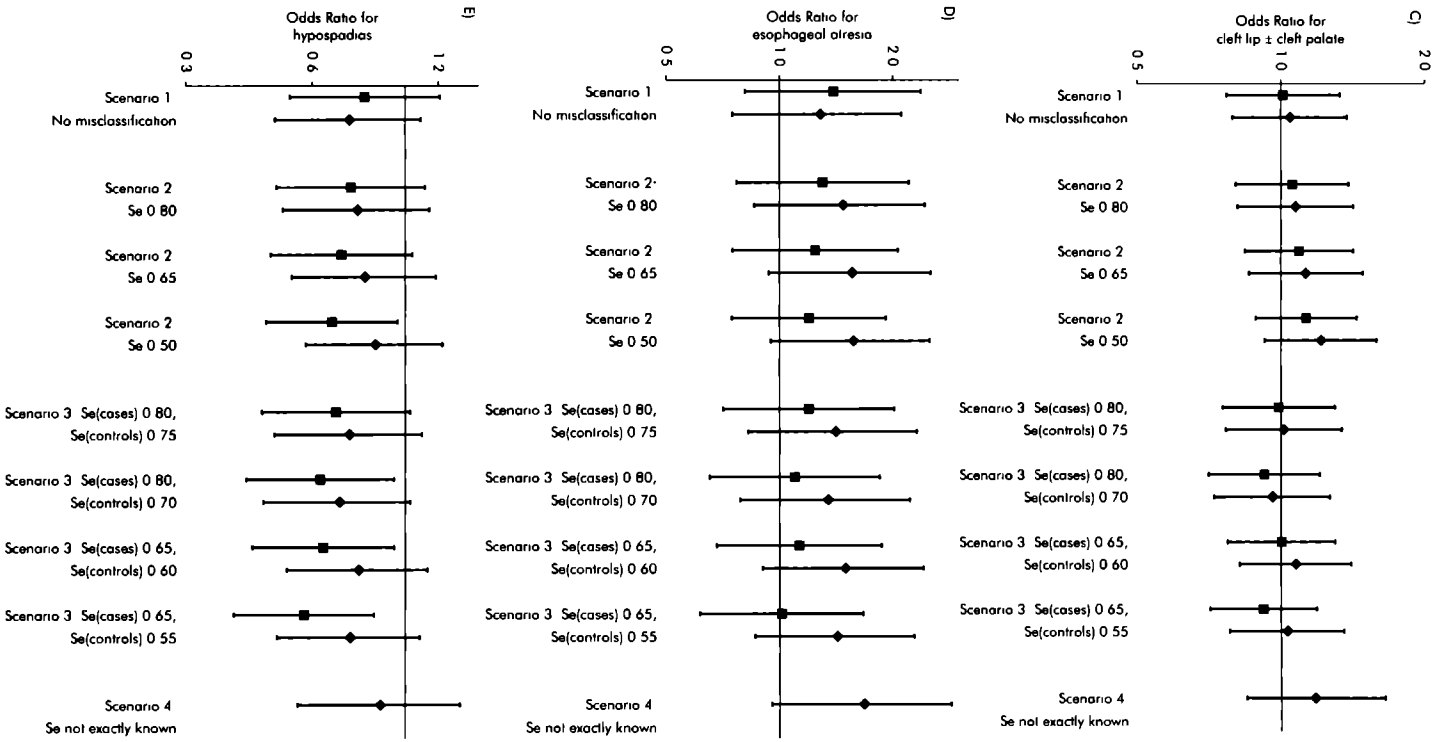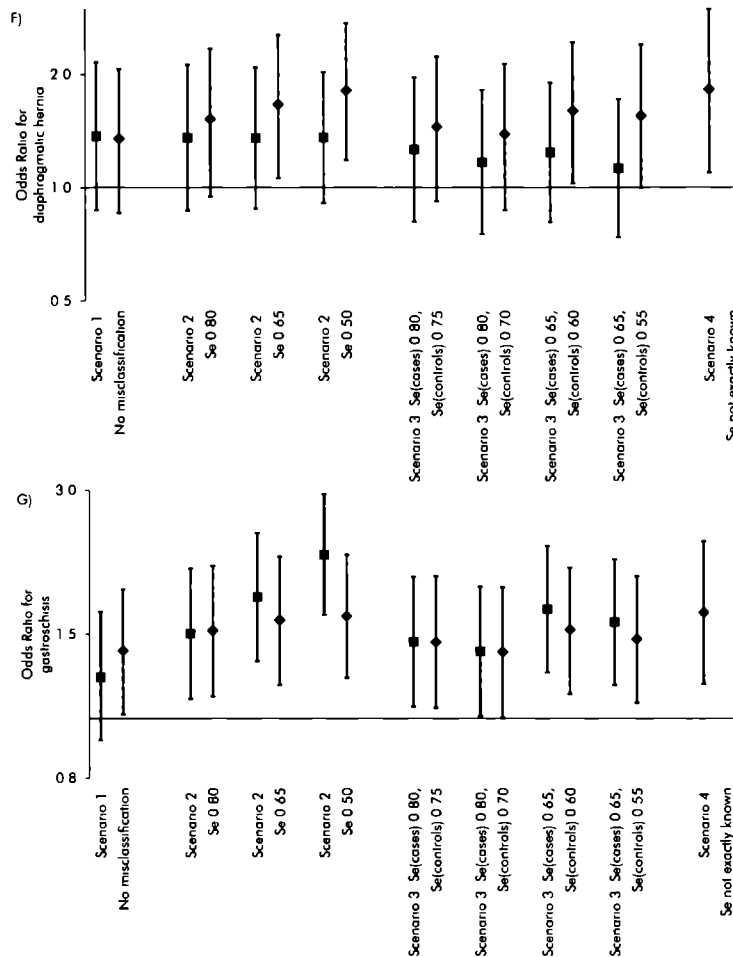
increased odds ratios after periconceptional cannabis use for anencephaly (OR 2.1, 95% CRI 1.2-3.6), esophageal atresia (OR 1.7, 95% CRI 1.0-2.9), diaphragmatic hernia (OR 1.8, 95% CRI 1.1-3.0), and gastroschisis (OR 1.7, 95% CRI 1.2-2.3).

The results of the sensitivity analyses indicated that placing vague priors on the coefficients in the outcome and exposure models did not change the results substantially (Appendix 10.5). However, the posterior ORs for the association between periconceptional cannabis use and anencephaly were slightly higher with wider posterior CRIs in the sensitivity analysis compared with the main analysis.

Discussion

After adjustments for exposure misclassification, we found associations between periconceptional cannabis use and the occurrence of anencephaly and gastroschisis in all Scenarios studied. In addition, increased odds ratios for esophageal atresia and diaphragmatic hernia were observed among infants exposed to cannabis in the periconceptional period in Scenarios 2 and 4 and in some cases of Scenario 3 when a Bayesian approach was used. The association with hypospadias seemed to change in the Monte Carlo simulations only.

The results from the Monte Carlo simulations and the Bayesian approach obtained in Scenario 1, which did not adjust for exposure misclassification, were very similar to the results reported previously.[14] In Scenarios 2 and 3, which assumed non-differential and differential misclassification, respectively, with a known value for sensitivity, results from the Monte Carlo simulations and the Bayesian approach were comparable for most defects. Differences in OR estimates between the two methods were observed for esophageal atresia, diaphragmatic hernia, and particularly gastroschisis, and may be caused by the fact that cannabis use is strongly associated with factors that were included as covariates in the outcome models.[31 33] For example, young maternal age is associated with both cannabis use and the occurrence of gastroschisis. In the Monte Carlo simulations, an adjusted cannabis exposure status was allocated to subjects categorized as non-exposed by the interview by applying false-negative probabilities that were equal for all subjects, regardless of maternal age. In contrast, differences in the probability of being exposed to cannabis between the age groups were taken into account in the Bayesian approach through the use of an exposure model. Therefore, the OR estimates produced by the Bayesian approach may be preferred over those produced by the Monte Carlo simulations.

In Scenario 4, which assumed that the sensitivities in the measurement model are unknown, the posterior ORs observed were larger than those produced in the other Scenarios, which is comparable with the pattern observed by MacLehose et al.[6] In addition to the associations between periconceptional cannabis use and anencephaly, diaphragmatic hernia, and gastroschisis that were also observed in other Scenarios, we found a borderline increased risk of esophageal atresia among infants exposed to cannabis in the periconceptional period in Scenario 4. However, because of the assumptions made and the fact that associations between periconceptional cannabis use and esophageal atresia and diaphragmatic hernia were not reported previously, these results should be interpreted with caution.

Except for the results for diaphragmatic hernia and gastroschisis, the OR estimates for the associations between periconceptional cannabis use and the majority of the

selected birth defects did not change considerably after adjustment for potential exposure misclassification, irrespective of the method used and even with sensitivities as low as 0.50. This may have been due to the relatively low exposure prevalence. The crude ORs clearly shifted further away from the null after adjustment for potential non-differential misclassification in the cannabis-gastroschisis association, which may be explained by the fact that reported periconceptional exposure to cannabis was far more common among mothers of infants in this birth defects category than among mothers of cases in the other birth defect groups. After correction for confounding, however, the analyses and in particular the Monte Carlo simulations showed that non-differential misclassification does not always lead to underestimation of the effect estimate.

Ideally, data obtained from a validation study conducted within the National Birth Defects Prevention Study should be used to quantify sensitivity and specificity of self-reported cannabis exposure status as these measures may vary across settings. However, due to the retrospective study design, we could only use external validation data, which were collected years before the start of the National Birth Defects Prevention Study in different populations (Table 10.1). Because participants in the validation studies were told about the testing procedures which may have increased reporting of exposure, we used somewhat lower values for sensitivity in our analyses than those observed in these studies. As a consequence, Scenario 4, which treated sensitivity as unknown, is of particular interest, although the choice for a prior distribution for this parameter may be debated. Moreover, we assumed that the specificity of the interview was 1.00 and that no measurement error was present in the confounding and outcome variables, so we cannot rule out that other types of error biased our results.

Exposure misclassification may have a serious impact on the validity of epidemiologic studies. The best solution is to measure exposures without error, but this is often impossible. Sensitivity analyses, such as the Monte Carlo simulations and the Bayesian approach presented in this paper, may provide insight into the possible impact of exposure misclassification on the effect estimates. Although Monte Carlo simulations are easier and less time-consuming to conduct, theoretically a Bayesian approach is preferable because of the use of an exposure model, which models the probability of being exposed conditional on a set of predictors. In practice, we observed only a few differences in the OR estimates between the two methods, which was recently suggested by others as well.[34] In the case of cannabis-birth defect associations, exposure misclassification may have obscured other possible associations, including those between periconceptional cannabis use and the occurrence of esophageal atresia, diaphragmatic hernia, and gastroschisis.

Furthermore, the analyses indicated that it is unlikely that the association between exposure to cannabis in the periconceptional period and anencephaly observed in the standard logistic regression analysis can be explained by exposure misclassification As stated previously,[6] it is doubtful that further case-control studies will be able to answer the question whether these associations are true or not without improvements in the methods of data collection

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention

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Appendix 10.1 R script for Monte Carlo Simulations.

```
simmisclas <- function(sensitivity) {
    astar <- sum((data$exposure == 0) & (data$outcome == 0), na rm = TRUE)
    bstar <- sum((data$exposure == 0) & (data$outcome == 1), na rm = TRUE)
    cstar <- sum((data$exposure == 1) & (data$outcome == 0), na rm = TRUE)
    dstar <- sum((data$exposure == 1) & (data$outcome == 1), na rm = TRUE)
    probDmin <- ((1-sensitivity)/sensitivity)*(cstar/astar)
    probDplus <- ((1-sensitivity)/sensitivity)*(dstar/bstar)
    data$exp <- ifelse(data$exposure == 1, 1, ifelse(data$outcome == 0, rbinom(length(data[,1]),
        1, probDmin), rbinom(length(data[,1]), 1, probDplus)))
    modraw <- glm(outcome ~ exp, family = binomial, data = data)
    coefraw <- coef(modraw)['exp']
    seraw <- sqrt(diag(vcov(modraw)))['exp']
    modcor <- glm(outcome ~ exp + confounders, family = binomial, data = data)
    coefcor <- coef(modcor)['exp']
    secor <- sqrt(diag(vcov(modcor)))['exp']
    (c(coefraw, seraw, coefcor, secor)) }
nsimul <- 10000
result <- t(replicate(nsimul,simmisclas(sensitivity)))
coefrawresult <- mean(result[,1])
serawresult <- sqrt(mean(result[,2] ^ 2) + var(result[,1]))
coefcorresult <- mean(result[,3])
secorresult <- sqrt(mean(result[,4] ^ 2) + var(result[,3]))
```

Appendix 10.2 Prior odds ratios (95% credible intervals) used in the outcome models.[a]

	Anencephaly	Perimembranous VSD	Cleft lip ± cleft palate[1,2]	Esophageal atresia[3,4]	Hypospadias[5,6]	Diaphragmatic hernia[3,7]	Gastroschisis[8]
Reported cannabis use in periconceptional period							
No (ref)	1 0	1 0	1.0	1.0	1.0	1.0	1 0
Yes	1 0 (0.2-5.0)	1 0 (0 2-5 0)	1.0 (0 2-5 0)	1 0 (0 2-5.0)	1 0 (0.2-5 0)	1 0 (0.2-5 0)	1.0 (0.2-5.0)
Maternal age at delivery[9,10]							
<25 years	1 5 (1.0-2 3)	1 0 (0.5-2 0)	1.2 (0.8-1 8)	1.0 (0 5-2.0)	1.0 (0.5-2.0)	1.0 (0.5-2.0)	3.5 (1 4-9.0)
25-34 years (ref)	1.0	1.0	1.0	1 0	1 0	1 0	1 0
≥35 years	1 0 (0.5-2 0)	1.0 (0.5-2.0)	1.0 (0 5-2.0)	1 6 (1 0-2.6)	1 5 (1 0-2.3)	1.3 (0 7-2 4)	0 4 (0 2-0 8)
Race or ethnicity[11,13]							
NH white (ref)	1.0	1.0	1.0	1 0	1 0	1 0	1.0
NH black	0.9 (0.6-1 4)	0.9 (0 4-2 0)	0 8 (0 4-1 6)	0 8 (0 4-1 6)	0 7 (0 5-1 0)	0.9 (0 5-1 6)	0.6 (0.3-1.2)
Hispanic	1.4 (1 0-2 0)	1.0 (0.5-2.0)	1.0 (0 5-2.0)	0.9 (0 5-1.6)	0 6 (0 4-1.0)	0 9 (0 5-1 6)	1 0 (0 5-2.0)
Other	1.0 (0.2-5 0)	1.0 (0 2-5 0)	1.0 (0 2-5 0)	1 0 (0 2-5 0)	0 9 (0 5-1 6)	1 0 (0 5-2.0)	1.0 (0.5-2.0)
Level of education[14]							
≤12 years	1 3 (0 9-1.9)	1 0 (0 2-5 0)	1 0 (0 5-2.0)	1 0 (0.5-2.0)	1 0 (0.5-2.0)	1.0 (0.5-2.0)	1.3 (0 7-2 4)
>12 years (ref)	1 0	1 0	1 0	1 0	1 0	1.0	1 0
Cigarette smoking in periconceptional period[15]							
No (ref)	1 0	1.0	1 0	1 0	1 0	1.0	1 0
Yes	1 0 (0 5-2.0)	1 0 (0.5-2.0)	1.4 (1.0-2.0)	1.0 (0.5-2 0)	1.2 (0.7-2.1)	1.0 (0.5-2.0)	1 6 (1 0-2 6)
Binge drinking in periconceptional period[15]							
No (ref)	1 0	1 0	1 0	1 0	1 0	1 0	1 0
Yes	1.0 (0.2-5.0)	1.0 (0.5-2.0)	1.0 (0.5-2 0)	1 0 (0.5-2.0)	1 0 (0 2-5.0)	1.0 (0 5-2 0)	1 0 (0.2-5 0)
Prepregnancy BMI[16,17]							
<30 (ref)	1.0	1.0	1.0	1 0	1 0	1.0	1 0
≥30	1.4 (1.0-2 0)	1 1 (0 6-2 0)	1 2 (0.8-1 8)	1.2 (0.9-1.6)	1 1 (0 7-1.7)	1.3 (0.9-1.9)	0.2 (0.1-0.4)
Periconceptional folic acid use[18]							
No (ref)	1.0	1 0	1.0	1 0	1 0	1 0	1.0
Yes	1.0 (0.5-2.0)	1.0 (0.5-2 0)	1 0 (0 5-2 0)	1 0 (0 5-2 0)	1 0 (0 5-2.0)	1.0 (0 5-2.0)	1.0 (0.5-2.0)

BMI, Body Mass Index, ref, reference group; VSD, ventricular septal defect

[a] The key studies (see below for complete reference) that were used to help inform prior knowledge are indicated in superscript.

Key references used to help inform prior knowledge:

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Appendix 10.3 R script and model specifications for Bayesian analyses.

```
## Required package:
library(BRugs)
## Check model file:
modelCheck("model.txt")
## Read data file:
modelData("data.txt")
## Compile model with 1 chain·
modelCompile(numChains=1)
## Read initial values:
modelInits("inits.txt")
## Burn in:
modelUpdate(1000)
## Specify the variables that should be monitored:
samplesSet(c("b1"))
## Run 19,000 additional iterations:
modelUpdate(19000)
## Summarize results:
samplesStats("*")
```

Codes for the misclassification models

The vector p contains prior means and pv contains 1/prior variance. Parameters a0, a1, ba1, and ba2 correspond to α_0, α_1, ba_1, and ba_2 in the text, respectively.

Model/Scenario 1

```
model {
    for (i in 1:N) {
        outcome[i] ~ dbern(pt[i])
        logit(pt[i])<-b0+b1*exposure[i]+b2*age_y[i]+b3*age_o[i]+b4*race_bl[i]+b5*race_his[i]+
        b6*race_oth[i]+b7*educ[i]+b8*smoke[i]+b9*binge[i]+b10*obese[i]+b11*fa[i]
        }
    b0 ~ dnorm(0,.000001)
    b1 ~ dnorm(p[1],pv[1])
    b2 ~ dnorm(p[2],pv[2])
    b3 ~ dnorm(p[3],pv[3])
    b4 ~ dnorm(p[4],pv[4])
    b5 ~ dnorm(p[5],pv[5])
    b6 ~ dnorm(p[6],pv[6])
    b7 ~ dnorm(p[7],pv[7])
    b8 ~ dnorm(p[8],pv[8])
    b9 ~ dnorm(p[9],pv[9])
    b10 ~ dnorm(p[10],pv[10])
    b11 ~ dnorm(p[11],pv[11])
    }
```
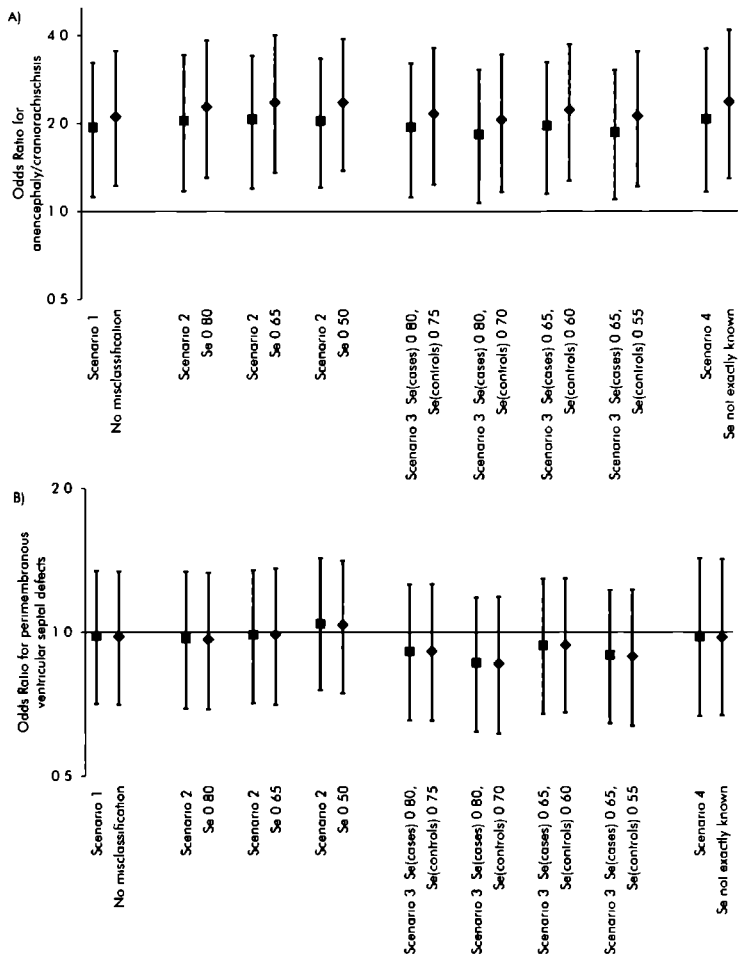
Models/Scenarios 2 and 3

```
model {
    for (i in 1 N) {
        outcome[i] ~ dbern(pt[i])
        logit(pt[i])<-b0+b1*exposure_s[i]+b2*age_y[i]+b3*age_o[i]+b4*race_b[i]+b5*race_his[i]+
        b6*race_oth[i]+b7*educ[i]+b8*smoke[i]+b9*binge[i]+b10*obese[i]+b11*fa[i]
            exposure[i] ~ dbern(exposure_a[i])
            exposure_a[i]<-a0*(exposure_s[i])*(1-outcome[i])+a1*(exposure_s[i])*outcome[i]
            exposure_s[i] ~ dbern(prop[i])
            logit(prop[i])<-g1+g2*age_y[i]+g3*age_o[i]+g4*race_b[i]+g5*race_hisp[i]+
            g6*race_oth[i]+g7*educ[i]+g8*smoke[i]+g9*binge[i]+g10*obese[i]+g11*fa[i]+
            g12*fcan1[i]+g13*fcan2[i]
    }
    b0 ~ dnorm(0, 000001)
    b1 ~ dnorm(p[1],pv[1])
    b2 ~ dnorm(p[2],pv[2])
    b3 ~ dnorm(p[3],pv[3])
    b4 ~ dnorm(p[4],pv[4])
    b5 ~ dnorm(p[5],pv[5])
    b6 ~ dnorm(p[6],pv[6])
    b7 ~ dnorm(p[7],pv[7])
    b8 ~ dnorm(p[8],pv[8])
    b9 ~ dnorm(p[9],pv[9])
    b10 ~ dnorm(p[10],pv[10])
    b11 ~ dnorm(p[11],pv[11])
    a0 <- sensitivity(controls)
    a1 <- sensitivity(cases)
    g1 ~ dnorm(0, 0001)
    g2 ~ dnorm(p[12],pv[12])
    g3 ~ dnorm(p[13],pv[13])
    g4 ~ dnorm(p[14],pv[14])
    g5 ~ dnorm(p[15],pv[15])
    g6 ~ dnorm(p[16],pv[16])
    g7 ~ dnorm(p[17],pv[17])
    g8 ~ dnorm(p[18],pv[18])
    g9 ~ dnorm(p[19],pv[19])
    g10 ~ dnorm(p[20],pv[20])
    g11 ~ dnorm(p[21],pv[21])
    g12 ~ dnorm(p[22],pv[22])
    g13 ~ dnorm(p[23],pv[23])
}
```

Model/Scenario 4

```
model {
    for (i in 1 N) {
        outcome[i] ~ dbern(pt[i])
        logit(pt[i])<-b0+b1*exposure_s[i]+b2*age_y[i]+b3*age_o[i]+b4*race_b[i]+b5*race_his[i]+
        b6*race_oth[i]+b7*educ[i]+b8*smoke[i]+b9*binge[i]+b10*obese[i]+b11*fa[i]
            exposure[i] ~ dbern(exposure_a[i])
            exposure_a[i]<-a0*(exposure_s[i])*(1-outcome[i])+a1*(exposure_s[i])*outcome[i]
            exposure_s[i] ~ dbern(prop[i])
            logit(prop[i])<g1+g2*age_y[i]+g3*age_o[i]+g4*race_b[i]+g5*race_hisp[i]+
            g6*race_oth[i]+g7*educ[i]+g8*smoke[i]+g9*binge[i]+g10*obese[i]+g11*fa[i]+
            g12*fcan1[i]+g13*fcan2[i]
    }
    b0 ~ dnorm(0, 000001)
    b1 ~ dnorm(p[1],pv[1])
    b2 ~ dnorm(p[2],pv[2])
    b3 ~ dnorm(p[3],pv[3])
    b4 ~ dnorm(p[4],pv[4])
    b5 ~ dnorm(p[5],pv[5])
    b6 ~ dnorm(p[6],pv[6])
    b7 ~ dnorm(p[7],pv[7])
    b8 ~ dnorm(p[8],pv[8])
    b9 ~ dnorm(p[9],pv[9])
    b10 ~ dnorm(p[10],pv[10])
    b11 ~ dnorm(p[11],pv[11])
    a0 ~ dbeta(ba1, ba2)
    a1 ~ dbeta(ba1, ba2)
    g1 ~ dnorm(0, 0001)
    g2 ~ dnorm(p[12],pv[12])
    g3 ~ dnorm(p[13],pv[13])
    g4 ~ dnorm(p[14],pv[14])
    g5 ~ dnorm(p[15],pv[15])
    g6 ~ dnorm(p[16],pv[16])
    g7 ~ dnorm(p[17],pv[17])
    g8 ~ dnorm(p[18],pv[18])
    g9 ~ dnorm(p[19],pv[19])
    g10 ~ dnorm(p[20],pv[20])
    g11 ~ dnorm(p[21],pv[21])
    g12 ~ dnorm(p[22],pv[22])
    g13 ~ dnorm(p[23],pv[23])
}
```

Appendix 10.4 Characteristics of mothers of infants with no major birth defects and case infants with selected birth defects Data from the National Birth Defects Prevention Study, 1997-2005.

Characteristic	Controls (n=6,556)		Anencephaly (n=329)			Perimembranous VSD (n=1,363)			Cleft lip ± cleft palate (n=1,735)			Esophageal atresia (n=419)		
	No.	%	No.	%	OR (95% CI)	No.	%	OR (95% CI)	No.	%	OR (95% CI)	No.	%	OR (95% CI)
Reported cannabis use in periconceptional period														
No (ref)	6,305	96	311	95	1.0	1,311	96	1.0	1,653	95	1 0	402	96	1.0
Yes	251	4	18	5	1.5 (0.9-2.4)	52	4	1 0 (0.7-1.4)	82	5	1 2 (1 0-1 6)	17	4	1.1 (0 6-1.8)
Maternal age at delivery														
<25 years	2,169	33	101	31	0.9 (0 7-1.1)	435	32	1.0 (0 9-1.2)	657	38	1.3 (1.1-1 4)	116	28	0 8 (0 7-1 1)
25-34 years (ref)	3,461	53	184	56	1.0	678	50	1.0	836	48	1 0	220	53	1 0
≥35 years	926	14	44	13	0 9 (0 6-1 3)	250	18	1 4 (1 2-1.6)	242	14	1 1 (0.9-1.3)	83	20	1 4 (1 1-1.8)
Race or ethnicity														
NH white (ref)	3,923	60	164	50	1.0	780	57	1.0	1,074	62	1.0	298	71	1.0
NH black	749	11	25	8	0.8 (0 5-1.2)	186	14	1 2 (1 0-1.5)	99	6	0 5 (0 4-0 6)	14	3	0.2 (0.1-0.4)
Hispanic	1,456	22	112	34	1.8 (1 4-2.4)	294	22	1.0 (0 9-1.2)	447	26	1 1 (1 0-1 2)	79	19	0 7 (0 6-0 9)
Other	400	6	26	8	1.6 (1 0-2 4)	96	7	1 2 (1 0-1 5)	110	6	1 0 (0 8-1.2)	28	7	0.9 (0 6-1 4)
Level of education														
≤12 years	2,697	41	165	50	1 4 (1 2-1 8)	585	43	1 1 (1 0-1.2)	838	48	1.3 (1.2-1 5)	148	35	0 8 (0 6-1 0)
>12 years (ref)	3,852	59	164	50	1.0	778	57	1.0	896	52	1 0	271	65	1.0
Cigarette smoking in periconceptional period														
No (ref)	5,358	82	290	88	1 0	1,100	81	1 0	1,310	76	1 0	346	83	1 0
Yes	1,197	18	39	12	0 6 (0 4-0 8)	262	19	1 1 (0 9-1.2)	425	24	1.4 (1.3-1.6)	73	17	0.9 (0 7-1.2)
Binge drinking in periconceptional period														
No (ref)	5,740	88	292	89	1.0	1,201	88	1.0	1,507	87	1 0	371	89	1 0
Yes	762	12	31	9	0.8 (0 5-1.2)	152	11	1.0 (0.8-1 1)	215	12	1 1 (0 9-1 2)	44	11	0 9 (0 6-1 2)
Prepregnancy BMI														
<30 (ref)	5,284	81	250	76	1 0	1,079	79	1.0	1,371	79	1 0	334	80	1 0
≥30	762	12	57	17	1 2 (0 9-1.6)	232	17	1.1 (1.0-1.3)	284	16	1 1 (0 9-1.3)	65	16	1.0 (0 8-1.3)
Periconceptional folic acid use														
No (ref)	3,196	49	157	48	1 0	675	50	1 0	909	52	1 0	178	42	1 0
Yes	3,360	51	172	52	1 0 (0 8-1 3)	688	50	1.0 (0.9-1 1)	826	48	0 9 (0 8-1.0)	241	58	1 3 (1 1-1 6)

Appendix 10.4 (Continued)

Characteristic	Controls (n=6,556)		Hypospadias (n=1,291)			Diaphragmatic hernia (n=498)			Gastroschisis (n=688)		
	No.	%	No.	%	OR (95% CI)	No.	%	OR (95% CI)	No.	%	OR (95% CI)
Reported cannabis use in periconceptional period											
No (ref)	6,305	96	1,259	98	1 0	473	95	1.0	606	88	1 0
Yes	251	4	32	2	0 6 (0 4-0.9)	25	5	1 3 (0 9-2 0)	82	12	3 4 (2.6-4 4)
Maternal age at delivery											
<25 years	2,169	33	292	23	0 6 (0 5-0.7)	154	31	0 9 (0 8-1 1)	542	79	6 4 (5 2-7 7)
25-34 years (ref)	3,461	53	726	56	1 0	266	53	1.0	136	20	1 0
≥35 years	926	14	273	21	1 4 (1 2-1.7)	78	16	1 1 (0 8-1 4)	10	1	0 3 (0 1-0 5)
Race or ethnicity											
NH white (ref)	3,923	60	923	71	1 0	312	63	1.0	366	53	1 0
NH black	749	11	165	13	0.9 (0.8-1 2)	38	8	0.6 (0.5-0 9)	54	8	0 8 (0 6-1.0)
Hispanic	1,456	22	110	9	0 3 (0.3-0.4)	112	22	1 0 (0 8-1 2)	212	31	1 6 (1 3-1 9)
Other	400	6	86	7	0.8 (0.6-1 1)	33	7	1.0 (0.7-1 5)	55	8	1.5 (1.1-2 0)
Level of education											
≤12 years	2,697	41	352	27	0.5 (0 5-0 6)	201	40	1 0 (0 8-1 2)	474	69	3.2 (2 7-3.8)
>12 years (ref)	3,852	59	939	73	1.0	296	59	1.0	211	31	1.0
Cigarette smoking in periconceptional period											
No (ref)	5,358	82	1,073	83	1.0	396	80	1 0	442	64	1 0
Yes	1,197	18	218	17	0 9 (0.7-1.0)	102	20	1 2 (0 9-1.4)	246	36	2 5 (2.1-2 9)
Binge drinking in periconceptional period											
No (ref)	5,740	88	1,135	88	1.0	435	87	1 0	538	78	1 0
Yes	762	12	140	11	0 9 (0 7-1 1)	61	12	1.1 (0 8-1.4)	141	20	2.0 (1 6-2 4)
Prepregnancy BMI											
<30 (ref)	5,284	81	1,054	82	1.0	387	78	1.0	643	93	1 0
≥30	762	12	211	16	1 1 (0 9-1 3)	91	18	1.2 (1 0-1.6)	30	4	0 2 (0.2-0.4)
Periconceptional folic acid use											
No (ref)	3,196	49	487	38	1 0	245	49	1.0	433	63	1.0
Yes	3,360	51	804	62	1.7 (1 4-1 9)	253	51	1.0 (0.8-1 2)	255	37	0.6 (0.5-0 7)

A)

(y-axis: Odds Ratio for anencephaly/craniorachischisis, ranging 0.5 to 4.0)

x-axis categories:
- Scenario 1 No misclassification
- Scenario 2 Se 0.80
- Scenario 2 Se 0.65
- Scenario 2 Se 0.50
- Scenario 3 Se(cases) 0.80, Se(controls) 0.75
- Scenario 3 Se(cases) 0.80, Se(controls) 0.70
- Scenario 3 Se(cases) 0.65, Se(controls) 0.60
- Scenario 3 Se(cases) 0.65, Se(controls) 0.55
- Scenario 4 Se not exactly known

B)

(y-axis: Odds Ratio for perimembranous ventricular septal defects, ranging 0.5 to 2.0)

x-axis categories:
- Scenario 1 No misclassification
- Scenario 2 Se 0.80
- Scenario 2 Se 0.65
- Scenario 2 Se 0.50
- Scenario 3 Se(cases) 0.80, Se(controls) 0.75
- Scenario 3 Se(cases) 0.80, Se(controls) 0.70
- Scenario 3 Se(cases) 0.65, Se(controls) 0.60
- Scenario 3 Se(cases) 0.65, Se(controls) 0.55
- Scenario 4 Se not exactly known

(Appendix 10.5 continues)

Appendix 10.5 Sensitivity analyses for placing vague prior distributions on all coefficients in the models: posterior odds ratios and 95% credible intervals for the association between periconceptional cannabis use and A) anencephaly/craniorachischisis, B) perimembranous ventricular septal defects, C) cleft lip ± cleft palate, D) esophageal atresia, E) hypospadias, F) diaphragmatic hernia, and G) gastroschisis, adjusted for exposure misclassification using Bayesian methods. Squares indicate the original estimates, rhombi those of the sensitivity analyses. Data from the National Birth Defects Prevention Study, 1997–2005. Se, sensitivity.

(Appendix 10.5 continues)

E)

Odds Ratio for
hypospadias

Scenario 1
No misclassification

Scenario 2
Se 0 80

Scenario 2
Se 0 65

Scenario 2
Se 0 50

Scenario 3 Se(cases) 0 80,
Se(controls) 0 75

Scenario 3 Se(cases) 0 80,
Se(controls) 0 70

Scenario 3 Se(cases) 0 65,
Se(controls) 0 60

Scenario 3 Se(cases) 0 65,
Se(controls) 0 55

Scenario 4
Se not exactly known

D)

Odds Ratio for
esophageal atresia

Scenario 1
No misclassification

Scenario 2
Se 0 80

Scenario 2
Se 0 65

Scenario 2
Se 0 50

Scenario 3 Se(cases) 0 80,
Se(controls) 0 75

Scenario 3 Se(cases) 0 80,
Se(controls) 0 70

Scenario 3 Se(cases) 0 65,
Se(controls) 0 60

Scenario 3 Se(cases) 0 65,
Se(controls) 0 55

Scenario 4
Se not exactly known

C)

Odds Ratio for
cleft lip ± cleft palate

Scenario 1
No misclassification

Scenario 2
Se 0 80

Scenario 2
Se 0 65

Scenario 2
Se 0 50

Scenario 3 Se(cases) 0 80,
Se(controls) 0 75

Scenario 3 Se(cases) 0 80,
Se(controls) 0 70

Scenario 3 Se(cases) 0 65,
Se(controls) 0 60

Scenario 3 Se(cases) 0 65,
Se(controls) 0 55

Scenario 4
Se not exactly known

Chapter 11.1

Web-based questionnaires: the future in epidemiology?

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Abstract

The traditional epidemiologic modes of data collection, including paper-and-pencil questionnaires and interviews, have several limitations, such as decreasing response rates over the last decades and high costs in large study populations. The use of Web-based questionnaires may be an attractive alternative but is still scarce in epidemiologic research because of major concerns about selective non-response and reliability of the data obtained. The authors discuss advantages and disadvantages of Web-based questionnaires and current developments in this area. In addition, they focus on some practical issues and safety concerns involved in the application of Web-based questionnaires in epidemiologic research. They conclude that many problems related to the use of Web-based questionnaires have been solved or will most likely be solved in the near future and that this mode of data collection offers serious benefits. However, questionnaire design issues may have a major impact on response and completion rates and on reliability of the data. Theoretically, Web-based questionnaires could be considered an alternative or complementary mode in the range of epidemiologic methods of data collection. Practice and comparisons with the traditional survey techniques should reveal whether they can fulfill their expectations.

Background

Systematic and thorough data collection plays an important role in every epidemiologic study, in which factors such as the characteristics of the target population, resources available, and sensitivity of the topic of interest determine the method of data collection chosen. Traditional approaches to gathering information from study subjects, including face-to-face and telephone interviews and paper-and-pencil questionnaires, increasingly fail to generate qualitatively good results within the financial parameters.[1] Participation rates in epidemiologic studies gradually decreased approximately 1% per year over the past decades, with even sharper declines in recent years.[2] A number of reasons for the growing rates of non-participation have recently been suggested, for example, a general decrease in volunteerism, higher demands for participation, oversurveying, and cell phone use.[3]

In the late 1990s, additional approaches to data collection using the Internet were introduced, including Web-based data entry and direct mailing of online questionnaires.[4,5] Because of the limitations of conventional survey modes and declining participation rates, a major impact on survey research was expected.[6] Indeed, Web-based questionnaires are now frequently used in psychological studies and marketing research, but their use in epidemiologic studies was only 1% in recently published articles (Table 11.1.1). Nevertheless, the Internet may become an important tool in epidemiologic data collection in the near future, especially for recruitment and follow-up of large cohorts. A few successful examples of this approach are already available, including the Millennium Cohort Study,[7] the Nurses and Midwives e-Cohort Study,[8] and the Danish Web-based Pregnancy Planning Study.[9]

Since Web-based questionnaires may be an attractive alternative to the traditional methods of data collection, epidemiologists need to become familiar with the possibilities and limitations of this relatively novel approach, especially since computer programmers instead of survey methodologists developed the various Web survey procedures.[10] In this article, we discuss the latest developments concerning the advantages and disadvantages of Web-based questionnaires and address some practical issues involved in applying Web-based questionnaires in epidemiologic research.

Table 11.1.1 Modes of data collection used in analytic epidemiologic research articles published in 7 high-impact general medical and epidemiologic journals in 2008-2009.[a]

Journal title	No. of analytic epidemiologic articles	Mode of data collection, %[b]				
		Interview	PPQ	WBQ	Other[c]	Unknown
General medical journals	1,314	21 2	22 6	1.6	89 8	1 2
British Medical Journal	310	27 4	34 8	1 9	78 1	0.0
Journal of the American Medical Association	319	23.5	22.3	3.1	89 3	0 3
Lancet	294	23.5	19 7	0 7	93 5	3 4
New England Journal of Medicine	391	12.5	15.3	0.8	96 7	1 3
General epidemiologic journals	780	38 5	40 3	0 9	82.8	1 0
American Journal of Epidemiology	455	40 0	43 5	0 2	84 4	0.7
Epidemiology	138	31 9	34.1	1 4	78 3	0 0
International Journal of Epidemiology[d]	187	39.6	36.9	2.1	82 4	2 7
Total	2,094	27 6	29.2	1.3	87 2	1.1

PPQ, paper-and-pencil questionnaire; WBQ, Web-based questionnaire.

[a] All research articles were assessed by two independent reviewers In case of inconsistencies, a third reviewer was consulted If only "questionnaire" was stated as method of data collection, we assumed a PPQ was used

[b] For some articles, multiple modes of data collection were used Therefore, the percentages do not add up to 100%

[c] Other modes include use of medical records or registries, (psychological) tests, and biological samples.

[d] Cohort profiles were also included

Advantages of Web-based questionnaires

Data collection using Web-based questionnaires generally improves data quality since validation checks can be incorporated with prompts that alert respondents when they enter implausible or incomplete answers Even without forced-choice formats, item non-response and "don't know" answers are reported to be less prevalent in Web-based questionnaires compared with postal questionnaires [11] Because data are entered electronically and may automatically be transformed into an analyzable format by common gateway interface (CGI) scripts,[12] errors in the process of data entry and coding are avoided as well Common gateway interface scripts can also be used to build in skip patterns to hide non-relevant follow-up questions, order questions randomly, give personalized feedback, or randomize participants to different versions of the questionnaire Visual and audio aids and pop-up windows providing additional information may be added to simplify responding, which is impossible in paper-and-pencil questionnaires However, all these additional features will increase download time, which may contribute to non-response [13]

Experience shows that Web-based questionnaires are returned more rapidly than postal questionnaires, with most respondents completing the questionnaire within a few days [14 15] Completing all questions in a Web-based questionnaire was estimated to take about half the time needed to answer the same number of questions in a telephone interview [16] Researchers are able to immediately adjust Web-based questionnaires to resolve unforeseen problems or to incorporate preliminary results or new developments [17] A data management system may be used to automatically send e-mail reminders and invitations for follow-up questionnaires to study participants, although follow-up of 'bounce-back' (undeliverable) e-mails will be time-consuming [18]

Although some authors state that the use of Web-based questionnaires results in substantial cost reductions,[19 21] others conclude that the cost savings are currently unknown [1 22] With Web-based questionnaires, costs for printing, postage, and data entry are avoided, but the set-up costs, including Web site and survey design, may be substantial, although the marginal costs for adding more participants to the study are relatively low [23] Therefore, the cost per response may be high when Web-based questionnaires are used in studies with small sample sizes or in populations with low response rates to Web surveys Studies that invited participants through e-mail reported cost benefits associated with using Web-based questionnaires [14 24 25]

Disadvantages of Web-based questionnaires

Two main disadvantages that may hamper the use of Web-based questionnaires in epidemiologic research were identified at the beginning of this century: (1) relatively high non-response rates compared with traditional modes of data collection and (2) concerns regarding the reliability and validity of the data obtained.[17,26] Reluctance to use Web-based questionnaires because of safety and confidentiality issues may also play a role.

Response rates

Response rates of less than 100% will lead to selection bias if the association between exposure and disease is different for participants than for all targeted subjects.[27] Self-selection is a common cause of selection bias,[28] but traditional modes of data collection have shown little bias resulting from nonparticipation.[29 30] Although higher response rates have been found in specific subgroups, such as the highly educated[25] and undergraduate students,[31] response rates for Web-based questionnaires have generally been lower than for postal questionnaires,[32,33] particularly when Web-based questionnaires first became available. However, since Internet access is rapidly increasing in developed countries (Table 11.1.2), the coverage differential is decreasing and will probably soon disappear for the most part. Recent studies have already shown that subjects responding to a Web-based questionnaire are comparable to those responding to traditional modes of data collection in terms of age, gender, income, education, and health status.[7,42,43] However, responders to Web-based questionnaires seem to be obese more often than responders to paper-

Table 11.1.2 Internet access in various developed countries in 2009

Country	Access to Internet in 2009		Growth 2000-2009,%[34]
	National statistics, %	Internet World Stats, %[34]	
Australia	74[35]	80 1	158 1
Canada		74 9	97 5
Denmark	86[36]	84 2	137 4
France		69.3	407 1
Germany	73[37]	65 9	126 0
Netherlands	93[38]	85.6	266.8
New Zealand		79 7	304.8
Norway	91ᵃ [39]	90 9	92 5
United Kingdom	76ᵃ [40]	76.4	203.1
United States	77[41]	74 1	138 8

ᵃ Used the Internet within the last three months

and-pencil questionnaires or national probability-based samples, possibly because of a more sedentary lifestyle [7 43]

Response to Web-based questionnaires may improve rapidly since recent studies reported that the overwhelming majority of respondents preferred the Web-based version to postal questionnaires and telephone interviews or had no preference [14 44 45] Many of the approaches known to increase response rates to postal questionnaires[46] are also applicable to Web-based questionnaires, but some methods cannot be used when study subjects are recruited electronically Sending a monetary incentive, for instance, is impossible, but providing nonmonetary incentives, such as lottery participation and survey results, may improve response rates significantly [47] Approaches specifically pertaining to Web-based questionnaires, including providing a PDF version[48] and careful use of design elements,[47] have shown some success in improving response rates Questionnaire length does not seem to influence response rates or the amount of missing data [49]

By automatically collecting so-called paradata or metadata, including date, time, and time to completion, Web-based questionnaires may provide useful insights into the answering process [50] These data could also be used to identify the best possible order of questions, which may substantially increase completion rates (i e the number of subjects who submitted the last page of the questionnaire divided by the number of subjects who agreed to participate) [51] When a multiple-page design is used, partial responses may be used to identify survey questions that were difficult to answer Subsequently, the researcher may adjust these questions, providing the opportunity to improve response rates and decrease item non-response

Reliability and validity of the data

For various reasons, including simple errors such as subjects' not scrolling down to find all questions or answering options, bad questionnaire design, and faster reading by Internet users, Web-based questionnaires were suspected of yielding larger amounts of measurement error than the traditional methods of data collection [17 52] The contrary seems to be true, however Studies in various areas of health research have shown that traditional epidemiologic risk factors can be collected with equal or even better reliability in Web-based questionnaires compared with traditional approaches The quality of data on anthropometry,[45] perceived health status,[53] oral contraceptive history,[44] smoking,[54] and alcohol use[55] was high when collected by Web-based questionnaires Agreement for dietary history assessed with a Web-based questionnaire was reported to be moderate,[44] but the repeatability and validity of Web-based dietary history questionnaires seem to be comparable to those of paper-and-pencil versions [56] Self-reported weight was shown to be a good proxy for weight

measured by a trained professional,[43] whereas health-related quality-of-life measures may reliably be collected using Web-based approaches as well.[57]

A number of instruments used for psychological and psychiatric clinical and research applications, such as the Edinburgh Depression Scale,[58] the Center for Epidemiological Studies Depression Scale,[59] the Kessler Psychological Distress Scale,[59] and the Obsessive Compulsive Inventory,[60] have been validated for administration via the Internet. However, Web-based administration may yield slightly different results compared with paper-and-pencil assessments.[59,61] Therefore, it is questionable whether all of the scores obtained online can be compared with offline cutoff scores.[62]

There are strong indications that Web-based questionnaires are less prone to social desirability bias than other methods of data collection,[63 65] which makes them very suitable for research on sensitive topics such as sexual behaviors, weight, and illicit drug use. Computers may produce a situation in which respondents feel more anonymous and private and less concerned about how they appear to others,[66] provided that they are alone when completing the questionnaire. Another prerequisite for obtaining less socially desirable answers is that the respondent be able to backtrack (i.e. adjust answers before submitting them).[67]

Application in epidemiologic research

Although many software packages, ranging from free-of-charge programs with very limited possibilities to purchasable but very extended packages, are available to create Web-based questionnaires, not all programs are suitable for epidemiologic research. Institutional review boards generally accept electronic data collection only if personal information is sent via a secure and encrypted connection and is stored behind a firewall, leaving most free and low-cost packages unsuitable for research purposes.

When a Web-based questionnaire is being created, many different issues may affect data quality and response rates. First of all, potential respondents use different hardware and software configurations, which will influence the presentation and thereby the validity of the questionnaire.[68] Secondly, decisions should be made about a one-page or a multiple-page design.[52] If a one-page design is used, all questions are presented on a single HyperText Markup Language (HTML) page. These questionnaires are identical for all participants and should be short, without complex skip patterns. Multiple-page designs, on the other hand, enable the application of, for instance, response validation, automatic skipping of questions,

and random question order, usually by using common gateway interface scripts at the end of each HTML page. Placing 4 to 10 questions on a single page is recommended to avoid scrolling, but doing so comes with a trade-off: fewer questions per screen increases data quality and respondent satisfaction but increases completion time.[69] When a questionnaire is presented on multiple pages, the respondent is not able to estimate the total length. As a solution, a progress indicator could be added, although it may decrease completion rates, especially in lengthy questionnaires.[70]

Other more subtle design features may influence data quality as well. Closed-ended questions requesting a single answer, for example, may be presented with radio buttons or dropdown lists. Whatever format is used, showing only the first few answer options should be avoided to prevent respondents from not looking at the other options.[71] For closed-ended questions that permit respondents to make multiple selections ("check all that apply"), matrices (a forced-choice format, in which respondents have to provide an answer for each item) are generally preferred over check boxes.[72] However, respondents should not be forced to choose arbitrary answers.

To ensure that answers to open-ended questions are in acceptable formats, error messages are often built into questionnaires. These messages, however, may increase respondent frustration and thereby decrease completion rates,[13] just as answer boxes of insufficient size do. Therefore, participants should be guided by visual elements to submit their answer in the desired format, such as by adjusting the size of the answer box to the number of digits expected, replacing the words "Month" and "Year" by "MM" and "YYYY" to reflect the desired number of digits, and placing the visual instructions in the natural reading path.[73] Definitions can be clarified in multiple ways, although making them always visible on the screen will result in the highest chance of their being read. If doing so is not possible, a rollover strategy, in which the definition is obtained by simply positioning the mouse pointer on the term, is preferred over clicking to open a separate window.[71]

Once a Web-based questionnaire has been created, participants can be recruited in two ways.[74] Subjects in the target population can be invited to participate in the study directly by general mail or e-mail, in which a link to the (password-protected) Web-based questionnaire is imbedded. Since a username is assigned to each individual with this approach, multiple entries from the same subject or questionnaire completions by others than the invited respondents are prevented and reminders may be sent to (partial) non-responders.[75] However, institutional review boards have been

reluctant to allow recruitment by e-mail, whereas typing in the Web address, login, and password offered in a letter may act as a barrier to participation.

Alternatively, as in the Danish Web-based Pregnancy Planning Study,[76] the questionnaire may be open to the public via recruitment strategies such as banners and advertisements. If this procedure is used, calculating a response rate is difficult and multiple completions from one participant cannot be prevented, although some strategies, such as recording Internet protocol addresses and personal data, may detect multiple submissions.[77] In addition, it is very likely that a selective population, whose characteristics are different from those of the target population, will participate when an open recruitment strategy is used,[21 78] which may limit its use in quantitative studies but may not be an issue in qualitative research.[79] Whatever recruitment strategy is used, informed consent will virtually always be required, via either Web-based or paper-based signed forms.

Conclusion

The current developments in the use of Web-based questionnaires as a mode of data collection in epidemiologic research are promising. They indicate that Web-based questionnaires, when carefully designed, could adequately be used in certain populations in developed countries, such as for college students and men and women of reproductive age. Because Internet access rates are rapidly increasing, the use of Web-based questionnaires should also be possible in other populations in the near future. Since Web-based questionnaires have scarcely been used in epidemiologic research so far, future studies in which this mode of data collection is used should determine the reliability of data obtained by this approach. Nevertheless, it should be kept in mind that no method of data collection is perfect. Theoretically, Web-based questionnaires are fully able to compete with traditional modes of data collection and should be considered as an alternative or complementary mode in the range of epidemiologic methods of data collection. In the coming years, practical application and comparison with more traditional survey techniques should reveal whether Web-based questionnaires can fulfill their expectations, but the first results look promising.

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Chapter 11.2

Reporting on the modes of data collection

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To gather more insight into the methods of data collection currently used in medical research, we analyzed research studies published in four high-impact general medical journals and three epidemiological journals in 2008-2009.[1] Two epidemiologists independently assessed the modes of data collection reported in each research paper.

Surprisingly, the proportion of inconsistencies between the two reviewers was high, especially for papers published in general medical journals (about 30%). Further examination revealed that these inconsistencies were mainly due to unclear reporting of the methods used, with phrases such as "Information was collected on [list of variables]", "Race/ethnicity was assessed by the investigator or study coordinator", and "Sociodemographic, clinical, treatment (...), and laboratory data are collected" without any specification. Did they use questionnaires or interviews, were any measurements taken, or was it all hearsay?

The choice of the method of data collection for a particular study depends on several factors, including, but not limited to, the type of study, sensitivity of the topic of interest, and costs of the measurements.[2] Valid measurement of exposures, outcomes, and potential confounders is essential in medical research to prevent biased results.[3] Since different methods of data collection yield various amounts of measurement error, detailed reporting on the methods used is of great importance to assess the quality of the study by both readers and reviewers or editors. Additionally, an adequate description of the methods of data collection used enables other research groups to replicate the original study.

In our view, researchers should improve the description of the modes of data collection used in their studies. In addition, we encourage medical journals to pay more attention to the way in which the methods are reported to improve the possibilities of critical appraisal.

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Chapter 12

Rationale and design of the PRegnancy and Infant DEvelopment (PRIDE) Study

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Abstract

To optimize the health of pregnant women and their children by evidence-based primary and secondary prevention, more scientific knowledge is needed. To overcome the methodological limitations of many studies on pregnancy and child health, which often use a retrospective design, we established the PRIDE (PRegnancy and Infant DEvelopment) Study. This is a large prospective cohort study that aims at including 150,000-200,000 women in early pregnancy to study a broad range of research questions pertaining to maternal and child health, preconception, prenatal, and perinatal care, and adverse developmental effects in offspring. Women are invited to participate by their prenatal care provider before or during their first prenatal care visit and are asked to fill out web-based questionnaires in gestational weeks 8-10, 17, and 34, as well as biannually throughout childhood until 21 years of age. In addition, a food frequency questionnaire and a paternal questionnaire are administered and medical records are consulted. Multiple validation studies will be conducted and paper-and-pencil questionnaires are available for women who cannot or do not want to participate through the Internet. For subgroups of participants, blood and saliva samples for genetic and biochemical analyses are being collected. Recruitment started in the first region on July 1, 2011 and will eventually cover all of the Netherlands. We expect that this study, which will be the largest birth cohort in the world so far, will provide new insights in the etiology of disorders and diseases that originate in pregnancy. The PRIDE Study is open for collaboration.

Background

Exposures that occur during gestation and early childhood may be associated with diseases and disorders that manifest themselves at birth, during childhood, or even later in life. Indeed, various associations between prenatal or early-life exposures and diseases that are typically diagnosed in childhood have been reported. For instance, maternal use of acetaminophen during pregnancy, *in utero* exposure to maternal smoking, and delivery by caesarean section have all been implicated to play a role in the etiology of childhood asthma.[1 3] Furthermore, increased rates of attention-deficit/hyperactivity disorder (ADHD) have been found after prenatal exposure to labetalol, preterm birth, and organophosphate exposure,[4 6] while gestational diabetes, vaginal bleeding, and neonatal jaundice may increase the risk of autism.[7 8] Numerous associations between early-life exposures and diseases that occur in adolescence or adulthood have been reported as well. Classical examples include associations between birth weight and the occurrence of ischemic heart disease,[9 10] obesity,[11] and diabetes.[12 13] However, the results of many of these studies focusing on early-life exposures and later diseases are inconsistent. Most likely, many risk factors for disorders such as birth defects, respiratory conditions, autism, ADHD, and childhood cancer, are as yet unknown. Identifying possible risk factors for these and other disorders is a crucial step in the development of preventive measures.

Prospective cohort studies may overcome many of the disadvantages of other basic epidemiologic study designs, although large numbers of participants are needed when relatively rare outcomes are studied. In a prospective study, selection and recall bias are minimized because exposure assessment takes place before the outcome is known. In addition, exposures can be measured in more detail and exposure assessment may be enhanced by taking biological and environmental samples at appropriate time points. By following subjects over time, causality may be addressed and temporal changes in various factors, such as maternal mental health and blood pressure, may be monitored. Therefore, birth cohort studies are recommended.

Existing birth cohort studies

As early as the 1950s, the value of birth cohort studies was acknowledged and two large studies, the California Child Health and Development Studies and the Collaborative Perinatal Project,[14 15] started enrolment at the end of that decade. To date, a total of 13 true longitudinal birth cohorts with at least 5,000 participants have been described which all enrolled women prospectively during pregnancy (Table 12.1).[14 32] Two of these included 100,000 women,[16 19] and a third is planning to do

Table 12.1 Overview of existing longitudinal birth cohort studies with at least 5,000 participants that enrolled women prospectively during pregnancy

Cohort	Location	Enrolment period	Timing of enrolment	Sample size	Reported response rate
Aarhus Birth cohort[24 25]	Aarhus, Denmark	Sept. 1989-	<16 weeks of gestation	>20,000	75%
ABCD Study[22]	Amsterdam, the Netherlands	Jan. 2003 -March 2004	First prenatal care visit	8,266	67%
ALSPAC[26 28]	Avon, England	April 1991-Dec 1992 (EDD)	Majority in early pregnancy	14,541	85%
Born in Bradford[29]	Bradford, England	March 2007-2010	Gestational weeks 26-28	10,000	NR
CCHDS[14]	California, USA	1959-1966	Early pregnancy	20,754	NR
CPP[15]	USA	1959	First prenatal care visit	60,000	NR
Danish National Birth Cohort[16 17]	Denmark	1996-2003	First prenatal care visit	100,000	30%
Generation R[27 30]	Rotterdam, the Netherlands	April 2002-Jan. 2006	76% early pregnancy; 21% mid-pregnancy, 3% late pregnancy	8,880	61%
HHf2[31]	Odense and Aalborg, Denmark	April 1984-April 1987	Gestational week 36	11,980	87%
Hokkaido Study[23]	Hokkaido, Japan	Feb. 2003-	<13 weeks of gestation	20,000	NR
National Children's Study[20 21]	USA	2009-	First trimester	100,000	NR
Northern Finland Birth Cohort[32]	Oulu and Lapland, Finland	1966 (EDD)	Gestational weeks 24-28	12,058	96%
Norwegian Mother and Child Cohort Study[18 19]	Norway	1999-2007	Gestational weeks 17-18	100,000	44%

ABCD, Amsterdam Born Children and their Development; ALSPAC, Avon Longitudinal Study of Parents and Children, CCHDS, California Child Health and Development Studies, CPP, Collaborative Perinatal Project; EDD, estimated date of delivery; HHf2, Healthy Habits for Two, NR, not reported.

so [20 21] Six birth cohorts enrolled women exclusively in the first trimester of pregnancy,[14 16 20 22 23] and three cohorts enrolled the majority of their participants in the first trimester [24 26 27] The reported response rates ranged from 30% to as high as 96% with a median response rate of 71% It seemed that the more recently conducted birth cohort studies had lower response rates than the earlier studies Regarding data collection, a variety of methods have been used (Table 12 2) In all cohorts, self-reported data were collected through questionnaires or interviews, but only four studies collected these data in all three trimesters of pregnancy [20 26 27] Most studies attempted to follow-up their cohorts into childhood Biological samples were mostly obtained from subgroups of participants only In addition, almost all birth cohort studies consulted medical or obstetric records to obtain clinical data and linkages to medical registries were often established In eight cohorts, mothers or infants were medically examined as well [14 15 20 22 26 27 29 32]

The existing birth cohort studies provide sufficient data to test a wide range of hypotheses, which already resulted in many research papers For example, by November 2011 over 500 research papers were published using ALSPAC data and 230+ papers were based on data from the Danish National Birth Cohort However, the existing birth cohorts also generate new hypotheses and subsequently pose new research questions which cannot be answered with the data collected, such as possible health risks associated with cell phone use and the effects of organic food consumption by either women or children In addition, common behaviors may change over time and may affect maternal, fetal, or infant health of this and future generations [33] To overcome the methodological problems associated with retrospective study designs and to test hypotheses that cannot be studied in the existing birth cohort studies because of power limitations or lack of sufficiently detailed data, we established a new prospective birth cohort study, the PRegnancy and Infant DEvelopment (PRIDE) Study

Goals of the PRIDE Study

We aim to include 150,000-200,000 Dutch women in early pregnancy in the PRIDE Study to evaluate a broad range of research questions pertaining to maternal and child health and adverse developmental effects in offspring The primary objective of the PRIDE Study is to identify factors to which women may be exposed during pregnancy that potentially affect the health of the future mother or her unborn child at any point in life Secondary aims of the PRIDE Study include (1) describing the distributions of potential risk factors during pregnancy and estimating incidences and prevalences of various common and relatively rare outcomes, and (2) evaluating

Table 12.2 Methods of data collection used in existing longitudinal birth cohort studies.

Cohort	Self-reported data			Biological samples		Other data sources
	Method	Timing prenatal	Postpartum until	Mother	Infant	
Aarhus Birth cohort	Q	Trimester 1	–	–	–	Medical records, registries
ABCD Study	Q	Early pregnancy	Adulthood	Blood	–	Medical records, registries, physical examinations
ALSPAC	Q	Multiple times	Adulthood	Blood, urine, placenta, hair, toe nail	Cord blood, umbilical cord, blood, urine, saliva	Medical records, environmental monitoring, home observations, educational records, physical examinations
Born in Bradford	I	Trimester 3	–	Blood, urine	Cord blood	Medical records, registries, physical examinations
CCHDS	I	Multiple times	Adolescence	Blood, placenta	–	Medical records, registries, physical examinations
CPP	I	Multiple times	–	Blood	–	Medical records, observations, physical observations
Danish National Birth Cohort	I	Multiple times	18 months	Blood	Umbilical cord	Registries
Generation R	Q	Multiple times	Adulthood	Blood, urine	Cord blood	Medical records, physical examinations
HHf2	Q	Trimester 3	–	–	–	Medical records
Hokkaido Study	Q	Trimester 1	School age	Blood, hair, breast milk	Cord blood	Medical records
National Children's Study	I	Multiple times	21 years	Blood, urine, placenta, breast milk, saliva, hair, vaginal swabs	Cord blood, umbilical cord, meconium, hair, blood, urine, saliva	Medical records, environmental monitoring, physical examinations
Northern Finland Birth Cohort	Q	Weeks 24-28	14 years	–	–	Medical records, registries, physical examinations
Norwegian Mother and Child Cohort Study	Q	Multiple times	7 years	Blood, urine	–	Registries

I, interview; Q, questionnaire.

specific aspects of preconceptional, prenatal, and perinatal care in the Netherlands (e g , counseling, screening, and prenatal diagnostic procedures)

Study design

Health care providers in prenatal care play a central role in the enrolment of pregnant women into the PRIDE Study They are contacted through the professional organizations of midwives, gynecologists, and general practitioners Participating health care providers give verbal and written information about the PRIDE Study to pregnant women and encourage them to visit the PRIDE Study website (www pridestudy nl) On this website, women can fill out the study questionnaires using a personal login code provided by their health care provider Basically all Dutch pregnant women are eligible for participation, the only two exclusion criteria are (1) maternal age less than 18 years, and (2) more than 16 weeks pregnant at intake Women are asked to participate on a completely voluntary basis and give informed consent digitally through the Internet or by regular mail In 2011, 99% of Dutch women aged 25-45 years had access to the Internet, 91% through a broadband connection [34] Paper-and-pencil consent forms and questionnaires are available for women who cannot or do not want to participate through the Internet In addition, every effort is made to improve response rates, including the careful use of design elements in the study questionnaires,[35] participation in monthly lottery drawings, and regular newsletters Reassuringly, the Danish National Birth Cohort and the Norwegian Mother and Child Cohort Study did not find indications for considerable bias in exposure-outcome associations resulting from non-participation [17 19] The PRIDE Study has been approved by the Regional Committee on Research involving Human Subjects for the first region Recruitment started here in July 2011 and will gradually be expanded to encompass all of the Netherlands in 2012 Inclusion is expected to be finished by the end of 2015

The complete structure of the data collection for the PRIDE Study is shown in Figure 12 1 In principle, pregnant women are invited to participate in the study by their midwife, gynecologist, or general practitioner through email and/or a regular letter just before or at their first prenatal care visit They are asked to complete Web-based questionnaires during gestational weeks 8-10 (etiologically relevant period for birth defects), 17 (before 20-week ultrasound), and 34 (just before delivery for most pregnancies), as well as biannually after giving birth until the infants reach the age of 21 years, starting with the first postnatal questionnaires two and six months after the expected date of delivery Specific questionnaires are available in case of a miscarriage or preterm birth In an extensive review of the literature, it was
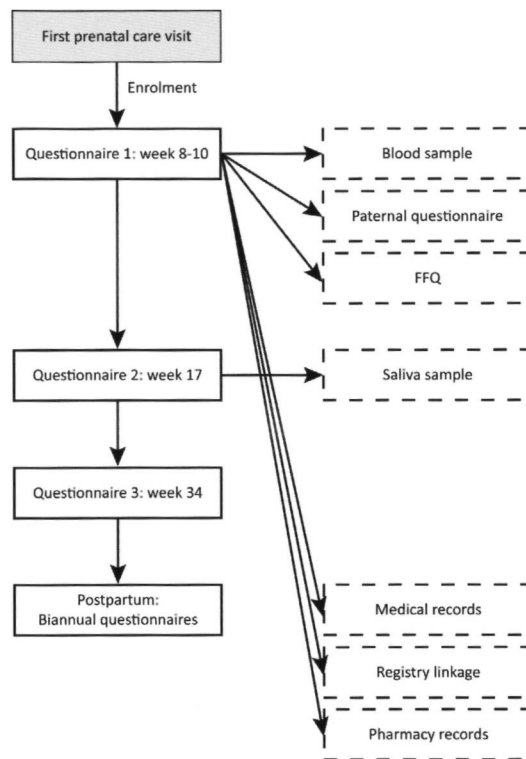
Figure 12.1 Structure of the data collection for the PRIDE Study. FFQ, food frequency questionnaire.

determined that this relatively new method of data collection is suitable for use among women of reproductive age.[36] Based on the results of this review, we incorporated some important measures in the data collection phase of the PRIDE Study to enhance data quality, such as the use of a mixed-mode design (Web-based and paper-and-pencil questionnaires) and initiation of various validation studies for data on, for instance, medication use and pregnancy complications. In collaboration with researchers from various medical specialties, including obstetrics, pediatrics, psychiatry, psychology, and physiology, existing paper questionnaires or parts thereof were selected, modified, and tailored to our Web-based application. Whenever possible, validated questionnaires and methods were used and incorporated into the PRIDE Study. In addition to the standard prenatal and postnatal maternal questionnaires, the women may fill out a detailed food frequency questionnaire around gestational weeks 8-10 and invite the future biological father to complete a questionnaire focusing on exposures in the three months before the index pregnancy.

In the informed consent form, permission is asked to consult pharmacy and medical records during pregnancy and after birth. If possible, we will also obtain data from the Netherlands Perinatal Registry, the National Institute for Public Health and the Environment, and the Comprehensive Cancer Center the Netherlands to verify specific exposures reported in the questionnaire, to collect more detailed information on both exposures and outcomes, and to assess the potential for bias resulting from non-participation.

Subgroups of participants, including those reporting a diagnosis of certain chronic conditions (e.g., depression, chronic hypertension) or use of selected drugs, such as antidepressants, antihypertensive medications, and statins, as well as all participants living in predefined geographic areas, are being invited to donate four 4.5 ml blood samples in the first part of pregnancy for genetic and biochemical analyses. These non-fasting blood samples are taken during routine blood sampling among pregnant women or by special invitation at any of the blood draw facilities of the Centers for Medical Diagnostics throughout the country. From three of the four blood samples, serum and plasma is being separated and subdivided into eight units (four serum, three plasma, and one erythrocytes); the fourth sample is whole blood for DNA extraction. All blood samples are stored at -80°C until laboratory analyses. To increase the numbers for future genetic analyses, women who are not included in blood sampling may later be asked to provide a saliva sample using DNA self-collection kits send by regular mail.

In the second prenatal questionnaire, women are also asked to donate a saliva sample to measure cortisol levels. The participating women are sent a polypropylene numbered tube by regular mail and are asked to collect a saliva sample within 10 minutes after waking up on a weekday. The samples are sent back by mail and subsequently stored at -20°C for biochemical analyses in nested case-control designs.

The PRIDE Study is not only designed for research on relatively rare exposures and outcomes, but also to study interactions between exposures including gene-environment interactions and the risks of more common diseases, such as asthma, autism, and ADHD. Although the PRIDE Study will initially include 150,000-200,000 pregnant women and permission for linkage with medical records and registries as well as exposure to many factors is assessed in the first prenatal questionnaire, we will not be able to conduct analyses using data from all subjects due to refusals, loss to follow-up, and missing values. Therefore, the power calculations are based on a conservative estimate of the size of our study population, namely 120,000, but if more subjects can be included, study power will increase. In the Netherlands, 177,713 infants were born in 2008, of which 7.7% were born preterm, 6.2% had a

low birth weight, and 2.8% had a major birth defect.[37] Therefore, the expected numbers of cases in our study population of 120,000 children are 9,240, 7,440, and 3,360 for preterm birth, low birth weight, and major birth defects, respectively. However, the number of infants with specific birth defects will be much lower, which limits the number of associations that can be studied with sufficient study power for these outcomes. Prevalences of diseases that manifest in childhood are less readily available for the Netherlands, but may be estimated using prescription rates for drugs used in the treatment of these diseases. Anti-asthma and ADHD medication have been prescribed to 4.9% and 2.1% of children, respectively,[38,39] corresponding to at least 5,880 and 2,520 children with asthma and ADHD in our study population. Using these figures, we calculated the minimal exposure prevalences needed to demonstrate a relative risk of at least 2.0 with a type I error of 5% and a type II error of 20%. The results of these calculations are shown in Table 12.3, which indicates that it will be possible to reliably study even rare exposures and combinations of exposures in relation to the development of various outcomes within the PRIDE Study.

Perspectives

From a public health perspective, it is of major importance to determine whether exposures that occur early in life are causally related to diseases and disorders that manifest themselves at birth, during childhood, or later in life. This scientific information may contribute to the implementation of evidence-based preventive measures for a large number of disorders, including birth defects, low birth weight, asthma, autism, ADHD, obesity, cardiovascular diseases, and diabetes. However, establishing a causal relation between intrauterine or childhood exposures and diseases that occur later in life is challenging, in particular since exposure to the factor of interest and to confounding factors may have taken place years or even decades before the outcome occurs.[40] Prospective birth cohort studies with large sample sizes may overcome many of the methodological shortcomings of cross-sectional and retrospective studies in perinatal and pediatric epidemiology.

Table 12.3 Minimal exposure prevalences needed to demonstrate a relative risk of ≥2.0 (α=0.05, study power 80%), based on 120,000 children

Outcome	Prevalence outcome	Expected no. of cases	Minimal exposure prevalence
Preterm birth	7 7%	9,240	0 08%
Low birth weight	6 2%	7,440	0 10%
Major birth defect	2 8%	3,360	0 23%
Asthma	4.9%	5,880	0.13%
ADHD	2.1%	2,520	0 31%

ADHD, attention-deficit/hyperactivity disorder

With a total of 150,000-200,000 pregnancies, the PRIDE Study will be the largest longitudinal birth cohort study conducted so far. Its prospective design in combination with the use of Web-based questionnaires allows us to measure a broad range of exposures in detail in etiologically relevant time frames. In addition, biomonitoring can be used to validate part of the self-reported data, while linkage with medical records and existing registries enables us to reliably collect clinical data on the health of participating mothers and children. This approach strongly increases data quality by minimizing the chance of information bias, especially when compared with retrospective study designs. We believe that this study will provide new and useful insights about the potential role of many prenatal and early-life exposures, such as medical drug use during pregnancy, fever and infections, maternal stress, pregnancy complications, diet, genetic factors, and parental occupational exposures, in the etiology of a large number of diseases. In the end, these insights may be used to improve maternal and child health by developing and implementing preventive measures in prenatal care and during childhood.

The PRIDE Study is open for collaboration with external groups. As recruitment and data collection are still ongoing, additional measurements may be implemented in the study if warranted. Requests for collaboration and proposals for projects should be sent to project@pridestudy.nl. Requests and proposals are discussed in the PRIDE Study Data Sharing Committee with respect to their study aims, feasibility, overlap with ongoing studies, and financial contributions. After approval by this committee and the Regional Committee on Research involving Human Subjects, a contract including mutual obligations will be drawn up and collaboration can commence.

Acknowledgements

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Chapter 13.1

General discussion
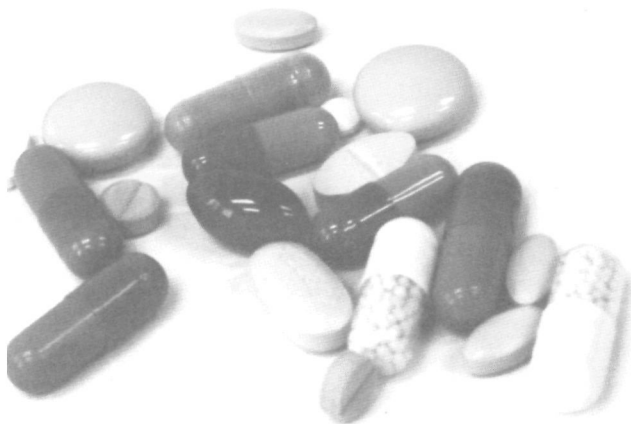
This thesis reflects the results of a research project in which we aimed to obtain more insight into the role of medical and illicit drug use during pregnancy in the etiology of specific birth defects and to evaluate the study methods that are frequently used in birth defects epidemiology. From the literature, it became clear that many medical drugs may be involved in the etiology of birth defects through various mechanisms, but also that epidemiologic studies on the teratogenic risks of these drugs are generally scarce (Chapters 2 and 3). In a validation study, we showed that the use of a self-administered questionnaire to assess prescription drug use during pregnancy in a retrospective study design may lead to considerable underreporting of use (Chapter 9.2). Using data from a large North-American case-control study based on interview data, we found associations between both treated and untreated hypertensive disorders during pregnancy and some specific birth defects, including septal defects, esophageal atresia, and hypospadias (Chapter 4). No associations were observed between exposure to non-steroidal anti-inflammatory drugs (NSAIDs) and selected birth defects in a prospective cohort study (Chapter 5). Regarding illicit drug use, we found that American cannabis and cocaine users engaged in many behaviors that may increase the risk of adverse reproductive outcomes (Chapter 6). Cocaine use in the periconceptional period seemed to increase the risk of cleft palate, but not the risk of the other birth defects studied. Cannabis use during pregnancy was not associated with preterm birth, low birth weight, or birth defects, with the notable exception of an increased risk of anencephaly (Chapters 7 and 8). Underreporting of the exposure of interest, however, may have obscured some cannabis-birth defect associations (Chapter 10). Web-based questionnaires were found suitable for use as a new method of data collection among men and women of reproductive age and may decrease exposure misclassification (Chapter 11.1). In the PRegnancy and Infant DEvelopment (PRIDE) Study described in Chapter 12, we incorporated Web-based questionnaires and other modes of data collection in a prospective study design to increase the reliability of the exposure data.

Before further discussing the details and implications of these results, some strengths and weaknesses of the study designs used in this research project will be addressed. Subsequently, the contribution of our studies to the field of medical and illicit drug use and major birth defects will be evaluated in light of the objectives of this thesis and recent findings of other studies. Lastly, the clinical and public health implications and directions for future research will be discussed.

Strengths and weaknesses of studies in birth defects epidemiology

Study population

Apart from the general methodological problems in epidemiologic research, such as confounding, selection and information bias, and limitations in making causal inferences, epidemiologic studies regarding risk factors for birth defects face some important problems that are unique to this area of research (Box 13.1.1).[1] Some of these are described in more detail here and in the following sections. Ideally, all individuals at risk for non-genetic structural birth defects, i.e. all conceptuses that are successfully implanted and survive to the embryonic period, are included in the denominator to describe incidence rates of these defects. In practice however, only prevalence rates of birth defects can be calculated at the time these defects become observable at mid-pregnancy ultrasounds or at birth. The prevalence rate is not a reliable estimate of the incidence rate as the proportion of malformed fetuses is higher among pregnancies ending in miscarriages or induced abortions. Even in prospective study designs it is impossible to identify all birth defects among the losses in clinically recognized pregnancies.[2]

Multiple gestations present a challenge for both the numerator and the denominator: should the study population consist of pregnancies or infants? To add complexity, the prevalence of birth defects is increased among multiple pregnancies compared with singleton pregnancies,[3] and including multiple pregnancies could lead to confounding or effect modification. In two of our studies (Chapters 4 and 5), we

Box 13.1.1 Challenges in birth defects epidemiology (adapted from Wilcox[1])

The following problems are not necessarily unique to birth defects epidemiology, but the combination of these conditions is typical for studies in this field:
• The true denominator for birth defects as an outcome is unknown.
• It is unclear whether the unit of analysis should consist of pregnancies or infants.
• Stillborn infants and terminations of pregnancy may have more severe types of birth defects than live born infants.
• Other pregnancy outcomes can compete with birth defects.
• Birth defects do not occur in isolation but are part of a continuum of development.
• Specific birth defects are rare outcomes.
• The quality of data on the diagnosis of specific birth defects varies between data sources.
• People who attempt to get pregnant at any given time are highly selected as past outcomes may influence future behavior.
• Birth defects usually involve parent-child triads instead of one person.
• The exact timing of exposure is extremely important.
• Data on reproductive issues are private and prone to social desirability bias.

therefore decided to exclude multiple gestations altogether. Alternatively, statistical models could be used that take this clustering into account, such as general estimating equations analysis,[4] the unit of observation could consist of pregnancies instead of infants, or, as was done in Chapters 8 and 10, only one of the infants could be included in the study population. In this case, the infant with the birth defect was included or the first-born infant if none or multiple infants born from the index pregnancy were affected.

In this research project, data from two large case-control studies, the Slone Birth Defects Study (BDS) and the National Birth Defects Prevention Study (NBDPS), were used. These multicenter studies conducted in the United States identify infants with birth defects and control subjects (live-born infants without birth defects) via birth defects registries, hospital discharge lists, and birth certificates.[5,6] Some but not all centers in both the BDS and NBDPS include stillborn infants and pregnancies that were terminated because of a prenatally diagnosed malformation, which may have more severe types of birth defects than live-born cases. Because the BDS includes very few terminations of pregnancy, we excluded these from our study population in Chapter 4, but they were included in the case group in our study on illicit drug use and birth defects (Chapters 8 and 10), which might have decreased the homogeneity of the birth defect groups. As there were relatively few terminations of pregnancy and stillborn cases, however, it is unlikely that including these cases affected our risk estimates substantially. Furthermore, the rates of termination of pregnancy did not differ between cannabis-exposed and non-exposed cases with anencephaly, our main outcome.

Study design and methods of data collection
Birth defects as a group are a very heterogeneous collection of disorders with each specific defect having its own set of risk factors, so they should not be considered as a single outcome.[7] However, the prevalence of specific birth defects is very low (as is shown in Table 3.2 in Chapter 3) and therefore, the case-control study is the most commonly applied design in epidemiologic studies of risk factors for birth defects.[8] When well-conducted, this study design is very efficient in estimating exposure-outcome associations provided that the exposure of interest is relatively common. With a 1:1 case:control ratio, 559 infants per group are required to detect an odds ratio (OR) of 2.0 if 5% of the controls are exposed (study power 80%, $\alpha = 0.05$). If the exposure prevalence drops to 0.5%, which is a more realistic estimate for use of specific medications and some illicit drugs, as many as 5,148 infants per group are required. The number of case infants in this scenario could be reduced to 2,958 if a 1:4 case:control ratio is applied and 11,832 controls were available. Therefore, the sample sizes of existing case-control studies limit the detection of weak to moderate

associations between specific medical and illicit drugs and the occurrence of specific birth defects as was the case in our studies described in Chapters 4, 8, and 10. Comparatively, even in very large cohort studies such as the Norwegian Mother and Child Cohort Study (Chapter 5) and the PRIDE Study (Chapter 12), only the most common birth defects can be studied. When the PRIDE Study will include 120,000 live births, stillbirths, and terminations of pregnancy, it will contain approximately 325 subjects with a ventricular septal defect, 100 with cleft lip ± cleft palate, and 25 with gastroschisis.[9] A successful method of increasing the sample sizes of both case-control and cohort studies in birth defects epidemiology is national and international collaboration.[10] In addition, methods that selectively enroll exposed subjects or two-stage designs, in which both exposed and affected subjects are oversampled, may be employed to increase their efficiency.[11]

In the NBDPS and BDS, cases were reviewed and coded according to established criteria by a clinical geneticist and an obstetric nurse practitioner, respectively, to increase the etiologic homogeneity of the case groups. In large cohort studies a review of the infants' medical records is not common practice, however. In the Norwegian Mother and Child Cohort Study (Chapter 5), data on birth defects were obtained through linkage with the Medical Birth Registry of Norway, in which the ascertainment of malformations varies according to their type and severity.[12,13] In addition, the lack of clinical details within medical birth registries often does not allow the researcher to compile homogeneous case groups. With a specificity of 100% and equal sensitivity among the exposed and non-exposed, misclassification of the outcome would not bias the risk ratio,[14] but study power will decrease. As these conditions are often not met, however, one should consider obtaining medical records from infants with and without birth defects, as identified by the birth registry, to decrease the level of outcome misclassification when conducting cohort studies.

Researchers are encouraged to use standardized definitions of birth defects to make comparisons between studies possible. For our systematic review (Chapter 3), we were forced to exclude some major defects, including hydrocephalus, renal agenesis, congenital hydronephrosis, and clubfoot, because their definitions varied between different studies. However, for pregnancy outcomes that are easier to ascertain, such as birth weight, gestational age, and mode of delivery, linkages to registries may be very efficient to obtain outcome data as long as they are checked for reporting inconsistencies (Chapter 7).

In birth defects epidemiology, timing of exposure is of major importance as the embryo is most vulnerable to teratogens in the first three to four months after conception. However, the specific embryonic structures develop in a certain sequence

and at particular stages during the embryonic period (Figure 13.1.1),[15] limiting the period in which the embryo is at risk for a specific structural birth defect to a period much shorter than three to four months. Most epidemiologic studies, including the studies in this thesis (Chapters 4, 5, and 8), define the exposure definition broader (e.g., one month before conception until the end of the third month of pregnancy) to take uncertainties in the date of conception and in the exact time period of exposure into account. This approach may introduce exposure misclassification if the actual exposure took place only before or after the sensitive period for the birth defect under study. Identifying the exact period of exposure is especially challenging in retrospective studies as the time lag between exposure and exposure assessment is relatively long. To decrease exposure misclassification, the BDS and NBDPS used a pregnancy calendar and completed their maternal interviews within six months and 24 months after the estimated date of delivery, respectively. Furthermore, a multilevel approach was used in the BDS to assess medication use. The women were first asked whether they had any of a list of specific illnesses and which drugs they used for these indications, followed by questions about use of medications for specific indications, and finally about use of medication identified by brand name (Chapter 4). This approach is far superior to the non-specific questionnaire validated in Chapter 9.2,[16] but may still result in exposure misclassification and is unsuitable for use in paper-and-pencil questionnaires due to its extensive lists of answers.

Although the recall interval is much shorter in prospective studies, suboptimal questionnaire designs can also yield exposure misclassification. In the paper-and-pencil questionnaire used in the Norwegian Mother and Child Cohort Study, medication use was assessed by illness, but only one line was printed to specify the drugs used to treat the specific condition and the time period of use. This made it impossible to determine which drug was used in which pregnancy week if multiple drugs were used in multiple pregnancy weeks. Therefore, we assumed that all medications were used in all pregnancy weeks selected, which may have led to non-differential misclassification in the study described in Chapter 5. However, when we excluded women with multiple drug exposure in multiple time windows (797 out of the 3,023 women reporting NSAID use) in a sensitivity analysis, the results did not change considerably.

In case-control studies of birth defects, recall of exposures may be biased by pregnancy outcome. Although there is evidence that recall bias is unlikely in this area of research,[17,18] its existence cannot be excluded. Therefore, 'malformed' controls (infants with birth defects other than the one of interest) or 'genetic' controls (infants with a genetic disorder) are sometimes used as the reference group. Indeed, in our

Figure 13.1 Period of vulnerability for selected birth defects. The dark shadings indicate the highly sensitive periods (adapted from Moore and Persaud[15]).
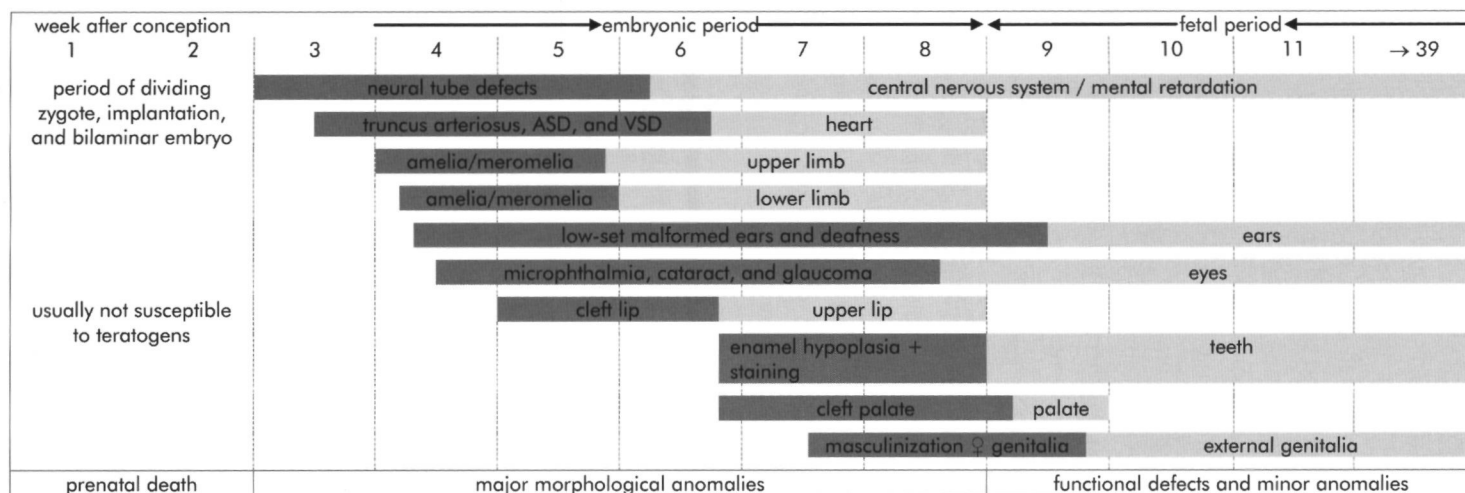
ASD, atrial septal defect; VSD, ventricular septal defect.

validation study (Chapter 9.2) we did not identify reporting differences between mothers of infants with non-genetic birth defects and mothers of infants with chromosomal or monogenetic disorders. However, using 'malformed' or 'genetic' controls could introduce selection bias or lead to underestimation of the risks if the controls have defects that are associated with the exposure of interest. In the epidemiologic studies in this thesis, only population controls were used as the reference group, so these problems were avoided, but recall bias may be present. As was shown in the simulation analyses on periconceptional cannabis use and birth defects (Chapter 10), however, the risks of anencephaly and gastroschisis remained increased after adjustment for relatively high levels of differential recall.

Specific issues pertaining to studying effects of medical drug use in pregnancy
In pharmacoepidemiology, the indication for use of a particular drug is probably the most important confounding factor as the reason for prescription (the underlying disease) may be associated with the outcome of interest.[19] This makes it very hard, if not impossible, to disentangle possible teratogenic effects of the medication from effects of the disease. During pregnancy, some diseases are only treated pharmacologically in severe cases, such as in hypertensive disorders and depression,[20,21] which complicates the interpretation of results even further. In Chapter 4, we attempted to deal with confounding by indication by focusing on both untreated and treated hypertensive disorders. Although higher risks were observed among treated women compared with untreated women for some birth defects, we cannot conclude that this is the result of exposure to antihypertensive drugs as the latter could also be a proxy measure for severity of the disease. This may especially have been the case when the onset of the hypertensive disorder occurred after the etiologically relevant time period for the birth defect of interest. Unfortunately, no data on the severity of the hypertensive disorders were available in the BDS to assess this issue in more detail. We were not able to address confounding by indication in Chapter 5 either, because most women who took a NSAID did not report the indication for use. However, as NSAIDs are used for a wide range of indications, we do not expect that this type of bias distorted our results to a great extent.

In addition to maternal self-report, prescription databases are sometimes used as data sources for prescription drug use in case-control and cohort studies as it may not be feasible to use self-reported modes of data collection in the study population selected. These databases may also provide more details on the drugs of interest than self-reported data, such as the dose prescribed, the amount dispensed, and the date of prescription. However, compliance to the medication dispensed varies among pregnant women with a generally high compliance to drugs for chronic conditions (70-100%), but a remarkably low compliance for local or short-time treatments (12-

77%).[22] Therefore, studies solely relying on prescription databases should be interpreted with caution as a dispensed prescription does not guarantee that the fetus was truly exposed. This is the case for our drug utilization study (Chapter 3), which may only give an indication of the prevalence of use of prescription drugs for which teratogenic mechanisms are known or suspected. Although theoretically possible, we did not assess compliance in our questionnaire validation study as it was beyond the scope of that paper (Chapter 9.2), but these data could be very valuable in estimating true exposure rates from prescription databases.

Specific issues pertaining to studying effects of illicit drug use in pregnancy
Collecting data on exposures to substances such as cannabis, cocaine, stimulants, hallucinogens, and heroin is accompanied by underreporting due to their illegal nature and social stigmas associated with use. In prospective studies, several biological specimens, including plasma, urine, hair, and amniotic fluid, can be used to identify prenatal illicit drug exposure.[23] The detection windows are usually small (1-3 days) with the exception of those for all drugs in hair and for cannabis in urine. Therefore, the time lag between exposure and data collection in case-control studies limits the use of biological specimens for exposure assessment as sampling has to be done exactly in the etiologically relevant time window to be of any value in birth defects epidemiology. That is why self-reported methods of data collection are virtually the only option to assess illicit drug use in retrospective studies, although medical records may provide some additional information. In our studies on illicit drug use and pregnancy outcomes (Chapters 7, 8, and 10), substantial underreporting is to be expected as personal interviewing yields relatively high degrees of social desirability bias compared with more impersonal methods of data collection.[24] Computer-administered modes, such as audio computer-assisted self-interviewing (ACASI, Chapter 6) and interactive voice response (IVR), could be used to increase the willingness to report sensitive information in face-to-face interviews and telephone interviews, respectively. Additionally, a Certificate of Confidentiality could be obtained in the United States to protect the confidentiality of the responses given.

As shown in Chapters 6 and 7, American subjects who report illicit drug use generally have a lower level of education and a lower household income than subjects who did not report use. Exactly these groups of people are underrepresented in epidemiologic studies due to lower participation rates.[25,26] In the National Survey of Family Growth (NSFG), weighted analyses were conducted to adjust for the different response rates to calculated unbiased estimates (Chapter 6). In our studies on the associations between illicit drug use and pregnancy outcomes based on data from the NBDPS (Chapters 7, 8, and 10), no adjustments for selective participation could be applied, although maternal education was indeed higher among participating controls than in

the source population.[27] It is unknown whether or in what way this potential selection bias influenced our results.

Illicit drug use is often accompanied by many other factors that may confound the associations between substance abuse and reproductive health or pregnancy outcomes.[28] Due to the descriptive nature of Chapter 6, we adjusted for a very limited number of potential confounders. Therefore, the ORs should be interpreted with caution as residual confounding may be present. However, as many other factors, including marital status and health status, were assumed to be intermediate factors instead of true confounders, adjusting for them would have introduced bias.[29] The limited number of exposed cases necessitated us to select only those factors that were expected to be the strongest confounders in Chapters 7, 8, and 10. Although residual confounding might be present, unnecessary adjustments resulting in imprecise estimates were avoided. Reassuringly, no indications for multicollinearity were observed even though some potential confounders, such as smoking and alcohol use, were strongly associated with illicit drug use.

The PRIDE Study: the ideal study design?
Within the prospective design of the PRIDE Study (Chapter 12), we attempted to tackle many of the methodological problems described above, in particular those related to exposure misclassification. The use of Web-based questionnaires enabled us to combine the advantages of paper-and-pencil questionnaires (self-assessment, low potential for social desirability bias) and interviews (possibilities for probing and use of extensive answering lists to collect very detailed data on, for example, medication use and occupational exposures), which will most likely result in high quality data. However, as Web-based questionnaires are a relatively new method of data collection in epidemiologic research (Chapter 11.1), multiple validation studies will have to be implemented. Paper-and-pencil questionnaires are available as an alternative, but so far less than 1% of the participants preferred this mode of data collection. Blood samples are collected very close to the etiologically relevant time window for birth defects, although few specific birth defects can be studied despite the large sample size when data collection is completed. When possible, we will not solely rely on maternal self-report for birth defect diagnoses and other health outcomes. Possibilities for linkage with the Netherlands Perinatal Registry and medical record review will be explored to limit outcome misclassification.

Returning to the objectives: what have we learned?

Medical drug use

In 2002, Lo and Friedman reported that the teratogenic risks in human pregnancy were undetermined for 91.2% of the 468 drug treatments approved in the United States between 1980 and 2000.[30] Their study was repeated in 2011, but the risk rating changed from "undetermined" to a specific risk assignment for only 16 of these drugs.[31] For the 172 drug treatments approved since 2000, the amount of data on the risks in human pregnancy was rated as "none" for 126 (73.3%) and as "very limited" for 33 (19.2%) drug treatments. We confirmed this lack of knowledge regarding prescription drugs for which teratogenic mechanisms are known or suspected in Chapter 3. It is cause for concern that the amount of research data available does not correspond with the prescription rates: the drugs most often dispensed in pregnancy were not necessarily the drugs most often studied. This becomes even more important since we and others found that the usage of some drugs among pregnant women, including antidepressants, vasoactive drugs, and anti-infective agents, is increasing over time.[32-34] Based on the relatively high first trimester prescription rates of antihypertensive drugs (7.6 per 1,000 pregnancies) and NSAIDs (21.4 per 1,000 pregnancies) in combination with few and conflicting results from previous studies on the teratogenicity, we selected these groups of drugs to be included in two hypothesis-testing epidemiologic studies (Chapters 4 and 5).

Several studies found associations between prenatal exposure to antihypertensive drugs, in particular angiotensin-converting enzyme inhibitors, and a number of birth defects,[35-37] but they also raised the hypothesis that the hypertensive disorder itself instead of its pharmacologic treatment may largely be responsible for the increased risks observed.[38,39] However, these studies were not able to address this possible confounding by indication as they could not differentiate the risks between the four clinical entities of hypertensive disorders during pregnancy. Since the pathophysiologic features of these entities are different,[40] one may expect that they affect the risk of birth defects differently, as has been observed for other pregnancy outcomes.[41] Indeed, we found increased ORs for some birth defects in relation to the specific types of hypertensive disorders (Chapter 4). For the increased risks associated with chronic hypertension and preeclampsia superimposed on chronic hypertension, which were observed irrespective of pharmacological treatment, a relatively simple etiologic model involving vascular disruption may be postulated (Chapter 2). This is not the case for the associations observed in relation to gestational hypertension and preeclampsia as these disorders by definition manifest themselves after the etiologically relevant time window for the birth defects studied. Although several hypotheses could be put forward, most likely subclinical states of gestational hypertension and preeclampsia may increase the risk of several cardiovascular

defects and severe hypospadias Generally, higher ORs for these birth defects were observed in relation to antihypertensive medication use, which may be a proxy measure for the severity of the disorder and earlier onset

Although NSAIDs are used frequently during pregnancy, few large-scale studies on their teratogenic risks have been conducted Our finding that NSAID use in the first 12 weeks of pregnancy did not seem to be a major risk factor for birth defects (Chapter 5) was confirmed by a study based on data from the NBDPS,[42] although in the latter small-to-moderately increased risks of several birth defects were observed in relation to exposure to some specific NSAIDs Due to the small numbers of cases for many specific birth defects in the Norwegian Mother and Child Cohort Study, we cannot definitely exclude increased risks for some of these birth defects However, as the overwhelming majority of human studies on the teratogenic risks of NSAIDs are reassuring, this is an example for the fact that results of animal studies, which found increased risks of cardiac, midline, and diaphragmatic defects,[43 44] are not always predictive for a teratogenic effect in humans Even if a drug is suspected to be teratogenic based on its mechanism of action (Chapter 2), it is anything but a guarantee that it is indeed involved in the etiology of birth defects However, the reverse may be true as well

The first objective of this research project was to assess the influences of medical drug use during pregnancy on the occurrence of major birth defects The studies described in this thesis point towards a tremendous lack of knowledge and the possibility that a large number of medical drugs may be teratogenic based on pathophysiologic mechanisms However, they also show that two groups of relative frequently used drugs, antihypertensive medication and NSAIDs, do not play an important role in the etiology of birth defects Possible contributions of the underlying maternal disorders should not be disregarded as these may confound study results in such a way that pharmacological treatment is unjustly identified as harmful for the developing fetus

Illicit drug use
Before the start of this project, many prejudices on the characteristics of pregnant women who use illicit drugs existed, but few were substantiated with scientific evidence Therefore, we tried to gain more insight in the reproductive health characteristics and sexual risk behaviors of men and women using drugs in the entire reproductive age range (Chapter 6) The specific aims of this study were (1) to identify potential confounders in studies on the associations between illicit drug use and pregnancy outcome, and (2) to identify groups of women who are at high risk of using illicit drugs based on their characteristics Although it was a cross-sectional study, the results indicated that cannabis and cocaine use is associated with many

factors that may affect pregnancy outcome, including age, race or ethnicity, education, and treatment for sexually transmitted diseases. Unfortunately, due to severe underreporting of unintended pregnancies and induced abortions in the NSFG,[45] we could not study the prejudice that illicit drug use is associated with unplanned pregnancies, which was one of the reasons why we included the months before pregnancy in our exposure definition in Chapters 8 and 10. Than *et al.*[46] found that women reporting unintended pregnancies were more likely to report illicit drug use than women who had intended pregnancies, which may indirectly justify our exposure window. Many of the factors identified in Chapter 6 were included as confounders in Chapters 7, 8, and 10. In addition, these factors were included in the exposure model in Chapter 10 to model the probability of true exposure to cannabis in the periconceptional period.

In line with most previous studies, we did not observe associations between cannabis use and low birth weight (<2,500 grams) or preterm birth (gestational age <37 weeks) in Chapter 7. In a prospective cohort study using medical records for exposure assessment, cannabis use during pregnancy was found to increase the risks of low birth weight (adjusted OR 1.7, 95% confidence interval (CI) 1.3-2.2) and preterm birth (adjusted OR 1.5, 95% CI 1.1-1.9).[47] However, Hayatbakhsh *et al.* did not adjust for gestational age in their low birth weight analyses and used a slightly different confounder set, which, in combination with the other methodological differences between the two studies, may explain the discrepancies in results. Hence, it is still unclear whether cannabis use during pregnancy is associated with low birth weight and preterm birth. Due to power limitations, we were not able to study associations between other types of illicit drugs and these pregnancy outcomes. However, the majority of previous studies focusing on prenatal cocaine exposure showed increased risks of low birth weight and preterm birth.[48] Other illicit drugs, such as ecstasy, methamphetamine, hallucinogens, and heroin, are scarcely studied with respect to birth weight and gestational age at birth, probably due to their low prevalence rates of reported use during pregnancy.

Although marijuana smoke contains substances that are strongly suspected to be teratogens,[49] and delta-9-tetrahydrocannabinol modulates the transcription of genes encoding for growth, cell morphology, and apoptosis in placental development,[50] cannabis use in early pregnancy does not seem to be associated with most birth defects. However, the results of our studies described in Chapters 8 and 10 indicate an increased risk of anencephaly, for which comparable results have been described in chick embryos.[51] After adjustment for exposure misclassification (Chapter 10), we observed increased odds ratios for esophageal atresia, diaphragmatic hernia, and gastroschisis as well. The latter was associated with cannabis use in a previous

study,[52] but the other associations represent new and unconfirmed findings. Periconceptional cocaine use may affect fetal development through vascular disruption[53] and has previously been associated with increased risks of a number of birth defects, including cardiovascular defects,[54 55] limb defects,[56] and gastroschisis,[57] but we could not confirm these findings in Chapter 8. Instead, we observed an increased risk of cleft palate, which is not one of the vascular disruption defects (Chapter 2). A variety of methodological differences, and possibly exposure misclassification, may account for these inconsistencies in results.

With respect to the second aim of this research project, which was to study associations between prenatal illicit drug exposure and major birth defects, our results were not consistent with previous studies, which found increased risks of other specific birth defects than we did. Therefore, this issue remains as yet unsolved. However, our studies on the characteristics of cannabis and cocaine users identified an important set of factors that should be included in future research on the teratogenic effect of these illicit drugs to decrease the likelihood of residual confounding.

Methodological considerations

It is generally acknowledged that exposure misclassification is a threat to the validity of epidemiologic studies and in particular case-control studies, which is the most commonly applied design in birth defects epidemiology.[8] In contrast to the measurement instruments used in other research areas, such as psychiatry and nutrition, many of the self-administered questionnaires and personal interviews used in prenatal and perinatal epidemiology have not been properly validated. The results of our validation study in Chapter 9.2 indicated that maternal recall of prescription drug use was moderate to poor in the existing questionnaire used, in particular for drugs used for short-term conditions. Comparable results were obtained in a validation study embedded in the Safety of Medications and Perception of Teratogenicity (SMART) study.[58] The level of agreement between self-report and prescription data was somewhat higher in the SMART study than in our study, which could be explained by the much shorter recall interval. In both questionnaires, a general screening question about prescription drug use was implemented, which was strongly advised against in previous reports.[16 59 60] Therefore, the validity of self-reported methods of data collection to assess prescription drug use may be higher if measurement instruments are developed more carefully, as has been done in the BDS and NBDPS. Nevertheless, inaccurate recall of prescription drug use and other exposures in early pregnancy cannot be excluded without well-conducted validation studies (Chapter 9.1).

As was shown in Chapter 10, underreporting may obscure existing associations and bias resulting from non-differential misclassification is not always towards the null value after correction for confounding. Although various methods are available to statistically adjust for exposure misclassification,[61-64] avoiding misclassification is preferable. As described in Chapter 11.1, use of Web-based questionnaires may improve reporting, which was confirmed by recent validation studies.[65,66] Due to the use of this novel method of data collection and shorter recall intervals, the PRIDE Study may produce high-quality data with which research questions pertaining to maternal and child health could be answered (Chapter 12). Web-based methods of recruitment and data collection are increasingly being used in reproductive epidemiology,[67,68] suggesting that these methods are gaining acceptability as alternatives to the more traditional modes of data collection.

The last objective of this research project was to evaluate the study methods, particularly the methods of data collection, frequently used in birth defects epidemiology. A self-administered questionnaire to assess prescription drug use during pregnancy performed poorly in a validation study and the resulting underreporting of exposure may obscure associations with birth defects. However, improvements in questionnaire design and use of Web-based questionnaires may decrease exposure misclassification and yield more valid results.

Implications for clinical practice, public health, and future research

The ultimate goal of etiologic research is primary prevention by avoiding exposures that may lead to detrimental health effects. Although the teratogenic risks of illicit drugs remain unknown, there is no doubt that exposure to these substances is harmful. Chronic cannabis use may possibly cause birth defects, but has definitely been linked to respiratory, cardiovascular, and psychiatric disorders.[69] Cocaine use clearly has an effect on general health, but during pregnancy it is not only associated with low birth weight and preterm birth,[48] but also with numerous other adverse outcomes, such as miscarriage, placental abruption, neonatal abstinence syndrome, and neurodevelopmental disorders.[70] Other illicit drugs, including opiates and amphetamine, have been associated with adverse pregnancy and developmental outcomes as well.[71-73] All things considered, even though the associations with birth defects are inconsistent, illicit drug use during pregnancy should strongly be discouraged in preconceptional and prenatal care visits, although in some situations controlled use may be preferred over abrupt discontinuation. As the implications are

much less straightforward for use of medication, the remainder of this Chapter will focus on medical drug use.

Many diseases among pregnant women, including epilepsy, diabetes, and severe hypertension and depression, require pharmacological treatment to benefit both maternal and child health. However, the current lack of knowledge on the teratogenic risks often hampers physicians in making evidence-based decisions on whether or not the beneficial effects of treatment outweigh the possible risks for the developing fetus. As the absence of evidence in combination with unproven allegations raise serious concerns, adequate counseling is necessary to decrease feelings of guilt and anxiety among pregnant women. This type of counseling should preferably start before conception, when adjustment in prescriptions could still be made to reduce possibly teratogenic exposures in early pregnancy, but requires a rapid increase of knowledge on teratogenic risks.

The current guidelines of the European Medicines Agency require pharmaceutical companies to prospectively collect at least 300 or 1,000 exposed pregnancies to reach the conclusion that the drug is not responsible for a 10-fold or 2-fold increase in the *overall* occurrence of congenital malformations, respectively.[76] A control group is not required when there are no reasons to believe that the indication for use may affect the birth defects prevalence and in that case, the prevalence of malformations is compared with the baseline prevalence of 3%. This approach has a number of drawbacks. Most importantly, weak or moderate associations between the medication and specific birth defects may be missed when only considering the overall occurrence of malformations, especially since monitoring 1,000 exposed pregnancies is by no means sufficient to detect increases in the prevalence of specific birth defects. In many pharmaceutical pregnancy registries, no control group is included because there is no evidence that the indication for use may be associated with birth defects, although the contrary has not been proven either. Furthermore, most registries rely on voluntarily reported exposures, which may lead to selection bias. Therefore, the current European guidelines for risk assessment may lead to the premature conclusion that a pharmacological treatment is safe for the developing fetus.

From a public health perspective, future research should focus on those medical drugs that are most commonly used during pregnancy and for which the teratogenic risks are unknown. Based on our results in Chapter 3, these could include iron preparations, serotonin receptor agonists or antagonists, drugs used in fertility treatment, and dihydrofolate reductase inhibitors. A focus on commonly used drugs in combination with drugs for which the largest effects may be expected would

provide the best opportunities to affect health and well-being of future generations. Even for medical drugs that are strongly associated with birth defects, such as thalidomide, isotretinoin, and certain anti-epileptic drugs, important research questions are still unanswered. For example, it is unknown why not all infants exposed to a teratogen in the etiologically relevant time window are affected. For thalidomide, risk estimates for birth defects range between 20% and 50% after exposure during the sensitive period.[74] Differences in dose or blood levels may account for these inconsistencies, but genetic factors could also contribute to variations in fetal susceptibility. With the use of pharmacogenetics, polymorphisms that affect teratogenic risks may be identified.[75] This could lead to the development of personalized or stratified medicine through which teratogenic drugs might safely offer unique or important therapeutic benefits for some pregnant women, if not for all.

In addition to mechanism-based studies intended to obtain insight in the teratogenic risks of medical drugs, well-conducted large-scale epidemiologic studies are required to improve counseling and prescribing patterns to finally enable reduction of the number of infants born with birth defects. In light of study power, case-control studies may seem the most logical choice, but problems associated with exposure assessment could bias their results to a great extent. A large international prospective cohort study could solve the power problems regarding birth defects that face the existing national or regional birth cohort studies. Preferable, such a study should not only rely on the traditional modes of data collection, but should also explore possibilities for real-time exposure assessment by means of, for instance, application software ('apps') on smartphones or tablet computers. Exposure assessment could include other factors that are suspected to play a role in the etiology of birth defects as well, such as nutritional factors, maternal stress, and occupational exposures.[7] In addition, when the follow-up extends beyond the neonatal period and continues into childhood, risk factors for other health outcomes that are thought to originate in pregnancy, such as asthma, autism, attention-deficit/hyperactivity disorder, some childhood cancers, and obesity, could also be studied. International prospective cohort studies aiming at decreasing the lack of knowledge regarding the risks of medical drug use during pregnancy should be initiated by consortia of international experts in the field of pharmacoepidemiology and reproductive epidemiology. Such a joint enterprise will only become reality with firm governmental and financial support at the level of for instance the European Union and the World Health Organization.

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Chapter 13.2
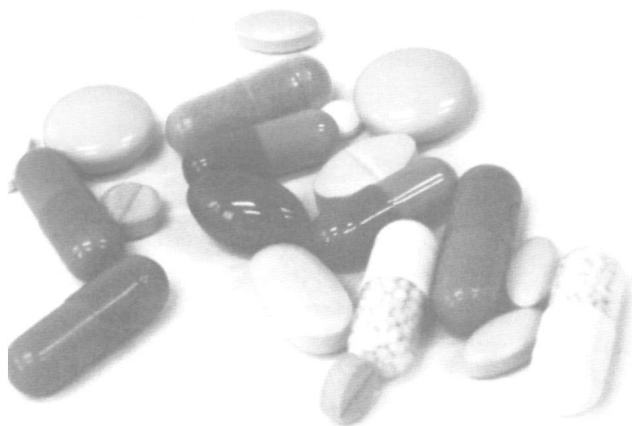
Summary

Major birth defects, defined as structural malformations that are of medical, surgical, or cosmetic importance, occur in approximately 2-3% of births and are among the main causes of infant mortality in the Western world. For the majority of birth defects the causes are as yet unknown, but both genetic factors and environmental exposures in the first three to four months of pregnancy have been implicated. Classic examples of human teratogens (non-genetic risk factors that cause birth defects) include medical drugs, such as thalidomide and isotretinoin. For more than 90% of prescription drug treatments, however, the human teratogenicity is undetermined in spite of the high prevalence of use among pregnant women. Furthermore, a substantial proportion of women use illicit drugs in the first part of pregnancy, but knowledge on their effects on fetal development is limited. This is partly due to the methodological challenges associated with studying illicit drug use during pregnancy.

The objectives of this research project, which are described in more detail in Chapter 1, were the following: (1) to assess the influences of medical drug use during pregnancy on the occurrence of major birth defects (Part I), (2) to study associations between prenatal exposure to illicit drugs and pregnancy outcomes including birth defects (Part II), and (3) to evaluate the study methods frequently used in birth defects epidemiology, focusing on the modes of data collection (Part III).

Part I: Medical drug use
In Chapter 2, we present a review of the literature conducted to identify mechanisms through which medical drugs may produce birth defects. Based on current knowledge from animal and human studies, six mechanisms were described: folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption, and specific receptor- or enzyme-mediated teratogenesis. Although it is preferable to study the teratogenic effects of specific drugs, these mechanisms may be used for research purposes to group medical drugs to increase study power. The pharmacological treatments suspected to be involved in these teratogenic mechanisms were studied in more detail in Chapter 3. First, we estimated their prescription rates among pregnant Dutch women using data from the IADB.nl database. In 17.7% of pregnancies in our study population, at least one drug associated with one of these teratogenic mechanisms was dispensed in the first trimester. Furthermore, the prescription rates in the first trimester increased over time for three drug groups, namely vasoactive drugs, selective serotonin-reuptake inhibitors (SSRIs), and serotonin receptor agonists/antagonists. Secondly, we conducted a systematic review of the literature to provide an overview of the current knowledge on the human teratogenic effects of the medical drugs studied. For a

number of drugs, including many antiepileptic drugs, antihypertensive medication, and some SSRIs, associations between exposure in early pregnancy and specific birth defects were observed in both cohort and case-control studies. However, for most drugs, too few exposed infants were studied to draw any conclusion regarding their human teratogenic potential.

Based on Chapters 2 and 3, we selected antihypertensive medication and non-steroidal anti-inflammatory drugs (NSAIDs) to be included in two hypothesis-testing epidemiologic studies. Using case-control data from the Slone Birth Defects Study in Chapter 4, we observed associations between the different types of hypertensive disorders and some birth defects, including associations between untreated chronic hypertension and esophageal atresia, preeclampsia superimposed on chronic hypertension and septal defects, untreated gestational hypertension and severe hypospadias, and untreated preeclampsia and severe hypospadias and ventricular septal defects. Furthermore, an association was observed for antihypertensive medication use for chronic hypertension and central nervous system malformations and for pharmacological treatment for gestational hypertension and ventricular septal defects and left-sided cardiovascular defects. Based on these findings and previous studies, we concluded that the hypertensive disorder itself, or its subclinical state, instead of the pharmacological treatment may be responsible for the associations observed.

Chapter 5 describes the results of the prospective cohort study in which the teratogenic risks of prescribed and over-the-counter NSAIDs were evaluated, using data collected in the Norwegian Mother and Child Cohort Study. NSAID use in the first 12 weeks of pregnancy did not seem to increase the risk of a number of specific birth defects, which were selected based upon results of previous studies and the proposed teratogenic mechanism of NSAIDs (inhibition of cyclooxygenases). However, due to the small numbers of cases in this study we could not definitively exclude increased risk for some specific birth defects.

Part II: Illicit drug use
In Chapter 6, we examined the reproductive health characteristics and sexual risk behaviors of American men and women from the entire reproductive age range who used cannabis or cocaine. We used data from the 2002 National Survey of Family Growth, and aimed at identifying potential confounding factors that could be used in future epidemiologic studies on illicit drug use and pregnancy outcome. In both young and older cannabis and cocaine users, drug use was associated with many factors that may affect pregnancy outcome, including age, race or ethnicity, level of education, and treatment for sexually transmitted and pelvic inflammatory diseases. The same sociodemographic factors were found to be associated with illicit drug use

among pregnant women in a study based on data from the control subjects enrolled in the U.S. National Birth Defects Prevention Study, described in Chapter 7. In that Chapter, we also evaluated the associations between cannabis use during pregnancy and birth weight and gestational age. After adjustment for confounding, no associations were observed between prenatal exposure to cannabis and these pregnancy outcomes.

In Chapter 8, we evaluated the teratogenic risks of periconceptional use of cannabis, cocaine, and stimulants again using data from the National Birth Defects Prevention Study. In this case-control study, we did not observe associations between exposure to these illicit drugs in the periconceptional period and most of the 20 birth defects categories included. Therefore, we could not confirm the associations observed in previous studies. However, in our study cannabis use seemed to be associated with an increased risk of anencephaly, while the risk of cleft palate was increased for infants exposed to cocaine, which both represent new and unconfirmed findings.

Part III: Methodological considerations

Exposure misclassification is a threat to the validity of epidemiologic studies and in particular case-control studies, which is the most common study design in birth defects epidemiology. Therefore, validation studies are necessary to ensure the quality of the data collected as stated in Chapter 9.1. In Chapter 9.2, we aimed to validate an existing questionnaire on prescription drug use during pregnancy. The reference standard was data collected by Eurocat Northern Netherlands, consisting of pharmacy records checked for compliance by maternal interviews. The validity of the self-administered questionnaire that was tested was moderate to poor for most drugs, in particular for drugs for occasional and short-time use. Furthermore, disagreement between the questionnaire and the reference standard was higher among women who smoked during pregnancy and when the questionnaire was completed more than two years after delivery. Therefore, future retrospective studies on medication use need additional data sources in addition to self-administered questionnaires and data collection should be completed as soon after delivery as possible.

Chapter 10 describes the potential influence of exposure misclassification on the cannabis-birth defects associations observed in Chapter 8. Multiple statistical methods are available to correct for potential biases resulting from misclassification. In this study, we applied relatively easy Monte Carlo simulations and a more sophisticated Bayesian approach. Few differences were observed in the odds ratio estimates between the two methods and they both showed that exposure misclassification may have obscured possible associations between periconceptional cannabis use and some birth defects, including esophageal atresia, diaphragmatic hernia, and gastroschisis. In addition, the association between cannabis use and

anencephaly observed in Chapter 8 could not be explained by non-differential or differential misclassification.

In Chapter 11.1, we summarized the literature on the advantages and disadvantages of Web-based questionnaires and some practical issues involved in implementing this relatively novel method of data collection in epidemiologic research. Although selection and information bias were previously assumed to limit the feasibility of Web-based questionnaires, more recent studies indicate that this method of data collection may be a valuable addition to the tradition modes of data collection, especially among men and women of reproductive age, and could even yield a higher data quality compared with interviews and paper-and-pencil questionnaires. Still, Web-based questionnaires are not often used in medical research, although their actual use might be underestimated due to poor reporting of the methods used (Chapter 11.2).

To overcome the methodological limitations of retrospective studies in prenatal and perinatal epidemiology, we established the PRegnancy and Infant DEvelopment (PRIDE) Study, for which we described the goals and design in Chapter 12. This prospective cohort study aims to include at least 150,000 women in early pregnancy to study a range of research questions pertaining to maternal and child health, preconception, prenatal, and perinatal care, and adverse developmental effects in offspring. The PRIDE Study will be the largest longitudinal birth cohort study conducted so far and is expected to provide insight in the role of many prenatal and early-life exposures in the etiology of a large number of diseases.

General discussion

In the General discussion in Chapter 13, several methodological issues concerning epidemiologic research on risk factors for birth defects were discussed, such as the composition of the study population, classification of specific birth defects and its resulting study power problems, and exposure assessment. Although a prospective cohort study such as the PRIDE Study may come close to the ideal study design in birth defects epidemiology, the expected numbers of cases with specific birth defects remains a limitation. We also summarized the contribution of our studies and the findings of other recent studies in light of the three objectives of this thesis. Finally, we discussed the implications of our studies for clinical practice, public health, and future research. We concluded that illicit drug use during pregnancy should be discouraged and that large-scale, international prospective cohort studies need to be initiated to obtain more insight in the role of medical drugs use in the etiology of birth defects and other diseases that originate in pregnancy.

Samenvatting

Ernstige aangeboren afwijkingen, gedefinieerd als structurele afwijkingen die medisch, chirurgisch of cosmetisch van belang zijn, komen bij ongeveer 2 à 3% van de geboortes voor. Ze behoren tot de belangrijkste oorzaken van kindersterfte in de Westerse wereld. Van de meeste aangeboren afwijkingen zijn de oorzaken nog onbekend, maar zowel genetische factoren als omgevingsfactoren in de eerste drie tot vier maanden van de zwangerschap zouden een rol kunnen spelen in de etiologie van aangeboren afwijkingen. Onder de schoolvoorbeelden van humane teratogenen (niet-genetische risicofactoren die aangeboren afwijkingen veroorzaken) bevinden zich een aantal medicijnen zoals thalidomide (Softenon) en isotretinoïne (Roaccutane). Ondanks het feit dat veel vrouwen voorgeschreven medicijnen gebruiken tijdens de zwangerschap, zijn de humane teratogene risico's nog onbekend voor meer dan 90% van deze behandelingen. Daarnaast gebruikt een substantieel deel van de vrouwen drugs in het eerste deel van de zwangerschap, maar de kennis over de effecten van deze stoffen op de foetale ontwikkeling is beperkt. Dit wordt deels veroorzaakt door de methodologische uitdagingen gerelateerd aan het bestuderen van drugsgebruik tijdens de zwangerschap.

De doelstellingen van het onderzoek beschreven in dit proefschrift zijn in detail uitgewerkt in Hoofdstuk 1. De doelstellingen waren: (1) het vaststellen van de effecten van medicijngebruik tijdens de zwangerschap op het vóórkomen van ernstige aangeboren afwijkingen (Deel I), (2) het bestuderen van verbanden tussen prenatale blootstelling aan drugs en negatieve zwangerschapsuitkomsten, waaronder aangeboren afwijkingen (Deel II) en (3) het evalueren van de onderzoeksmethoden die vaak gebruikt worden in epidemiologisch onderzoek naar aangeboren afwijkingen, met name de methoden van dataverzameling (Deel III).

Deel I: Medicijngebruik

Hoofdstuk 2 bevat een uitgebreide literatuurstudie om de mechanismen te identificeren waarlangs medicijnen aangeboren afwijkingen zouden kunnen veroorzaken. Gebaseerd op kennis verkregen uit dierexperimentele studies en epidemiologisch onderzoek zijn zes mechanismen beschreven: foliumzuur-antagonisme, verstoring van de neurale lijstcellen, verstoring van de hormoonhuishouding, oxidatieve stress, verstoring van de bloedvoorziening en activatie of remming van specifieke receptoren en enzymen. Ondanks dat het bestuderen van de teratogene effecten van specifieke medicijnen de voorkeur heeft, kunnen deze mechanismen gebruikt worden om in wetenschappelijk onderzoek medicijnen te groeperen om zo de *power* te verhogen. De voorgeschreven

medicijnen waarvan gedacht wordt dat ze betrokken zijn bij de zes teratogene mechanismen zijn in detail bestudeerd in Hoofdstuk 3. Met behulp van data uit de IADB.nl database hebben we geschat hoe vaak deze medicijnen verstrekt worden aan Nederlandse zwangere vrouwen. In 17,7% van de zwangerschappen in onze onderzoekspopulatie werd in het eerste trimester tenminste één medicijn dat geassocieerd is met een van de teratogene mechanismen verstrekt. Voor drie medicijngroepen, namelijk vasoactieve medicijnen, specifieke serotonineheropname-remmers (SSRI's) en agonisten en antagonisten van serotoninereceptoren, nam het percentage zwangerschappen waarin het medicijn in het eerste trimester verstrekt werd toe tijdens de onderzoeksperiode (1998-2007). Vervolgens hebben we een systematisch literatuuronderzoek gedaan om de huidige kennis over de teratogene eigenschappen van deze medicijnen bij de mens samen te vatten. Voor een aantal medicijnen, waaronder vele anti-epileptica, antihypertensiva en enkele SSRI's, werden verbanden tussen blootstelling vroeg in de zwangerschap en specifieke aangeboren afwijkingen gezien in zowel cohort- als patiënt-controle onderzoeken. Voor de meeste medicijnen zijn echter te weinig blootgestelde kinderen beschreven in de literatuur om conclusies te kunnen trekken over hun teratogene potentieel bij mensen.

Naar aanleiding van de resultaten beschreven in Hoofdstuk 2 en 3 hebben we antihypertensiva en prostaglandinesynthetaseremmers (NSAID's) geselecteerd voor twee hypothesetestende epidemiologische studies. In Hoofdstuk 4 vonden we met data uit de Slone Birth Defects Study, een Noord-Amerikaans patiënt-controle onderzoek, verbanden tussen de verschillende vormen van hypertensieve aandoeningen en een aantal aangeboren afwijkingen, waaronder associaties tussen onbehandelde chronische hypertensie en oesophagusatresie, chronische hypertensie met gesuperponeerde pre-eclampsie en septumdefecten, onbehandelde zwangerschapshypertensie en ernstige hypospadie en onbehandelde pre-eclampsie en ernstige hypospadie en ventrikelseptumdefecten. Ook werd er een verband gevonden tussen het gebruik van antihypertensiva voor chronische hypertensie en afwijkingen aan het centrale zenuwstelsel en tussen farmacologische behandeling voor zwangerschapshypertensie en ventrikelseptumdefecten en afwijkingen aan de linkerkant van het hart. Gebaseerd op deze bevindingen en voorgaande onderzoeken hebben we geconcludeerd dat de associaties die gevonden zijn niet veroorzaakt worden door de farmacologische behandeling, maar door de hypertensieve aandoening zelf of door een subklinisch voorstadium daarvan.

Hoofdstuk 5 beschrijft de resultaten van een prospectief cohortonderzoek waarin de teratogene risico's van bepaalde voorgeschreven en vrij verkrijgbare ontstekings-remmers/pijnstillers (NSAID's) geëvalueerd zijn met behulp van data uit de

Norwegian Mother and Child Cohort Study. NSAID gebruik in de eerste 12 weken van de zwangerschap leek het risico op de geselecteerde aangeboren afwijkingen niet te verhogen. Deze afwijkingen waren geselecteerd op basis van de resultaten van voorgaande onderzoeken en het mogelijke teratogene mechanisme van NSAID's (remming van cyclo-oxygenases). Door het relatief kleine aantal blootgestelde kinderen met aangeboren afwijkingen in dit onderzoek kunnen we echter verhoogde risico's voor een aantal specifieke aangeboren afwijkingen niet uitsluiten.

Deel II: Drugsgebruik

In Hoofdstuk 6 hebben we de karakteristieken met betrekking tot de reproductieve gezondheid en risicovol seksueel gedrag bestudeerd van Amerikaanse mannen en vrouwen in de reproductieve leeftijd die cannabis of cocaïne gebruikten. We hebben hiervoor data gebruikt van de 2002 National Survey of Family Growth met als doel potentieel verstorende factoren te identificeren die gebruikt kunnen worden in toekomstig epidemiologisch onderzoek naar drugsgebruik en zwangerschaps-uitkomst. Onder zowel jonge als oudere cannabis- en cocaïnegebruikers was drugsgebruik geassocieerd met vele factoren die de zwangerschapsuitkomst kunnen beïnvloeden, zoals leeftijd, ras of etnische achtergrond, opleidingsniveau en behandeling voor seksueel overdraagbare aandoeningen of ontstekingen in het kleine bekken bij vrouwen. Dezelfde sociaal-demografische factoren bleken geassocieerd te zijn met drugsgebruik door zwangere vrouwen in een studie die gebaseerd was op de controlepopulatie van de Amerikaanse National Birth Defects Prevention Study, die beschreven is in Hoofdstuk 7. In hetzelfde Hoofdstuk hebben we ook gekeken naar verbanden tussen cannabisgebruik tijdens de zwangerschap en geboortegewicht en zwangerschapsduur. Na correctie voor verstorende factoren vonden we geen associaties tussen prenatale blootstelling aan cannabis en deze zwangerschapsuitkomsten.

In Hoofdstuk 8 hebben we de teratogene risico's van periconceptioneel gebruik van cannabis, cocaïne en stimulantia geëvalueerd door opnieuw gebruik te maken van data van de National Birth Defects Prevention Study. In dit patiënt-controle onderzoek vonden we geen verbanden tussen het gebruik van deze drugs in de periconceptionele periode en het merendeel van de 20 categorieën aangeboren afwijkingen die bestudeerd zijn. Hierdoor konden we de verbanden die gevonden waren in voorgaande studies niet bevestigen. In ons onderzoek leek cannabisgebruik echter wel geassocieerd te zijn met anencefalie en was het risico op een gehemeltespleet verhoogd voor kinderen die prenataal blootgesteld waren aan cocaïne. Dit zijn beiden nieuwe bevindingen die nog niet bevestigd zijn in andere studies.

Deel III: Methodologische overwegingen

Misclassificatie van de blootstelling is een bedreiging voor de validiteit van epidemiologisch onderzoek. Dit geldt in het bijzonder patiënt-controle onderzoek, het onderzoeksontwerp dat het meest gebruikt wordt in epidemiologisch onderzoek naar risicofactoren voor aangeboren afwijkingen. Zoals beschreven in Hoofdstuk 9.1 zijn validatiestudies daarom noodzakelijk om de kwaliteit van de verzamelde data te garanderen. In Hoofdstuk 9.2 hebben we een bestaande vragenlijst over het gebruik van voorgeschreven medicijnen tijdens de zwangerschap gevalideerd. De 'gouden standaard', verzameld door Eurocat in Noord Nederland, bestond uit apotheek-gegevens die door middel van interviews met de moeder gecontroleerd waren voor therapietrouw. De validiteit van de onderzochte schriftelijke vragenlijst was matig tot slecht voor de meeste medicijnen, in het bijzonder voor medicijnen voor tijdelijk en kortdurend gebruik. Daarnaast bleek dat discrepantie tussen de vragenlijst en de 'gouden standaard' vaker voorkwam bij vrouwen die rookten tijdens de zwanger-schap en wanneer de vragenlijst meer dan twee jaar na de bevalling ingevuld was. Daarom zouden toekomstige retrospectieve onderzoeken naar medicijngebruik naast vragenlijsten aanvullende informatiebronnen moeten gebruiken en dient de dataverzameling zo snel mogelijk na de bevalling plaats te vinden.

Hoofdstuk 10 beschrijft de potentiële invloed van misclassificatie van de blootstelling op de verbanden tussen cannabis en aangeboren afwijkingen die gevonden waren in Hoofdstuk 8. Er zijn diverse statistische methoden beschikbaar om voor mogelijke vertekening door misclassificatie te corrigeren. In dit onderzoek hebben we zowel relatief eenvoudige Monte Carlo simulaties en een meer verfijnde Bayesiaanse benadering toegepast. Er waren weinig verschillen tussen de resultaten van de twee methoden. Beiden lieten zien dat misclassificatie van de blootstelling verbanden tussen periconceptioneel cannabisgebruik en een aantal aangeboren afwijkingen, waaronder oesophagusatresie, hernia diaphragmatica en gastroschisis, gemaskeerd kan hebben. Ook bleek dat het verband tussen cannabisgebruik en anencefalie dat gevonden was in Hoofdstuk 8 niet verklaard kan worden door differentiële of niet-differentiële misclassificatie.

In Hoofdstuk 11.1 hebben we de literatuur over de voor- en nadelen van digitale vragenlijsten en een aantal praktische zaken omtrent het gebruik van deze relatief nieuwe methode van dataverzameling in epidemiologisch onderzoek samengevat. In het verleden werd aangenomen dat selectie- en informatiebias het gebruik van digitale vragenlijsten beperken, maar meer recente onderzoeken tonen aan dat deze methode een waardevolle aanvulling op de traditionele methoden van dataverzameling kan zijn, vooral bij onderzoek onder mannen en vrouwen in de reproductieve leeftijd. Daarnaast werd duidelijk dat data die verzameld zijn met deze

methode van hogere kwaliteit kunnen zijn dan data verkregen door middel van interviews of papieren vragenlijsten. Desondanks worden digitale vragenlijsten niet vaak gebruikt in medisch wetenschappelijk onderzoek, maar het gebruik kan onderschat zijn doordat de gebruikte methoden vaak onnauwkeurig gerapporteerd worden (Hoofdstuk 11.2).

Om de methodologische beperkingen van retrospectief onderzoek op het gebied van prenatale en perinatale epidemiologie te ondervangen zijn we van start gegaan met de PRegnancy and Infant DEvelopment (PRIDE) Study. De doelen en de onderzoeksopzet van deze studie zijn beschreven in Hoofdstuk 12. In dit prospectief cohortonderzoek proberen we minstens 150.000 vrouwen vroeg in de zwangerschap te includeren om vele onderzoeksvragen op het gebied van de gezondheid van moeder en kind, preconceptionele, prenatale en perinatale zorg en nadelige effecten op de ontwikkeling van het nageslacht te beantwoorden. De PRIDE Study zal het grootste longitudinale geboortecohort worden tot nu toe en we verwachten dat deze studie inzicht zal verschaffen in de rol van vele blootstellingen tijdens de zwangerschap en op kinderleeftijd in de etiologie van een groot aantal aandoeningen.

Algemene discussie

In de Algemene discussie in Hoofdstuk 13.1 worden verschillende methodologische zaken met betrekking tot epidemiologisch onderzoek naar risicofactoren voor aangeboren afwijkingen besproken, zoals de samenstelling van de onderzoeks-populatie, de classificatie van specifieke aangeboren afwijkingen en de bijbehorende *power*-problemen en het vaststellen van de blootstelling. Hoewel een prospectief cohortonderzoek zoals de PRIDE Study in de buurt komt van het ideale ontwerp voor epidemiologisch onderzoek naar aangeboren afwijkingen, blijft het te verwachten aantal kinderen met specifieke aangeboren afwijkingen toch een beperking. Ook hebben we in dit Hoofdstuk de bijdrage van onze onderzoeken en de bevindingen van andere recente studies samengevat in relatie tot de drie doelstellingen van dit proefschrift. Tenslotte zijn de implicaties van ons onderzoek voor de klinische praktijk, de volksgezondheid en toekomstig onderzoek besproken. We hebben geconcludeerd dat drugsgebruik tijdens de zwangerschap ontmoedigd moet worden en dat grootschalige, internationale prospectieve cohortonderzoeken opgezet zouden moeten worden om meer inzicht te krijgen in de rol van medicijngebruik in de etiologie van aangeboren afwijkingen en andere aandoeningen die ontstaan tijdens de zwangerschap.

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Ook al verliep de opzet van de PRIDE Study niet helemaal volgens schema, ik ben erg blij dat de dataverzameling nu loopt en dat de beschrijving van dit onderzoek toch nog deel uitmaakt van mijn proefschrift. Dat was zeker niet mogelijk geweest zonder de bijdrage van de deelneemsters en de verloskundigen en gynaecologen die meewerken aan deze studie of zonder de inbreng van collega's. Reini, ik vind het jammer dat je de start van de dataverzameling niet meer als coördinator meegemaakt hebt. Maar jouw invloed is nog steeds duidelijk zichtbaar in het hele onderzoek! Bedankt! Jolt, Chris en Peter, jullie enthousiasme en ideeën hebben de PRIDE Study duidelijk versterkt en ik ben ervan overtuigd dat dit in de toekomst ook zeker tot mooie resultaten gaat leiden. PMD International bedank ik voor de ontwikkeling van het web-based systeem voor de dataverzameling. Daarnaast wil ik ook Michelle, die als stagiaire de folder mee ontwikkeld heeft, en Eline, Jolinde en Steffanie, die als student-assistent hulp geboden hebben bij de opzet en uitvoering van de PRIDE Study, bedanken.

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About the author

Marleen van Gelder was born in Nijmegen on January 27th, 1983. After graduating from secondary school ('gymnasium') at the Titus Brandsmalyceum in Oss in 2001, she studied Biomedical Sciences at the Radboud University Nijmegen. In 2004, she obtained her Bachelor of Science degree with honors and in 2006, she received her Master of Science in Biomedical Sciences with a major in Epidemiology. She conducted her Master internship of eight months at the National Center for Birth Defects and Developmental Disabilities of the Centers for Disease Control and Prevention (CDC) in Atlanta, USA (supervision by dr. J. Reefhuis and dr. N. Roeleveld. After graduating in December 2006, Marleen worked as a junior researcher on risk factors for male subfertility and testicular cancer at the Department of Epidemiology, Biostatistics and HTA of the Radboud University Nijmegen Medical Centre. She obtained a NWO Toptalent grant, with which she started to work as a PhD student and teacher at the same department in September 2007. The results of this PhD project on the effects of medical and illicit drug use during pregnancy on the occurrence of birth defects are described in this thesis. During this project, she spent six weeks at the School of Pharmacy in Oslo, Norway, one month at the CDC in Atlanta, USA, and one month at the Slone Epidemiology Center at Boston University, USA. In 2010, she received a 'Frye Stipend' for talented female researchers from the Radboud University Nijmegen. Marleen was awarded the Heinz Berendes International Travel Award 2011 from the Society for Pediatric and Perinatal Epidemiologic Research and a scholarship from the International Society for Pharmacoepidemiology in 2011. She has continued her work as epidemiologist and coordinator of the PRIDE Study at the Department of Epidemiology, Biostatistics and HTA focusing on medication use during pregnancy. Marleen lives with her partner Hans in Nijmegen.

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PhD theses Human Reproduction, NCEBP

2000

1   07-02-2000   Els van der Molen
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2007

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Human subfertility explorative studies on some pathophysiologic factors in semen and follicular fluid

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The transmission of chromatin and DNA lesions by sperm and their fate in the zygote (1)

26   01-11-2007   Godfried van der Heijden
The transmission of chromatin and DNA lesions by sperm and their fate in the zygote (2)

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On the measurement of recovery following hysterectomy

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Proton magnetic resonance spectroscopy of human fetal brain

2008

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Clinical encounters Dynamics of patient-centred practices in a Dutch fertility clinic

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IVF and Internet

2009

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Implementation of single embryo transfer

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IVF in the Netherlands success rates, lifestyle, psychological factors and costs

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44   06-06-2012   Marleen van Gelder
*The role of medical and illicit drug use in the etiology of birth defects Epidemiologic studies and
methodological considerations*

The role of medical and illicit drug use in the etiology of birth defects

Epidemiologic studies and methodological considerations

1. Gezien de hoge prevalentie van blootstelling is het onverantwoord dat de kennis over de risico's van medicijngebruik tijdens de zwangerschap zo beperkt is. [dit proefschrift]

2. Niet alleen de risico's van medicijngebruik tijdens de zwangerschap, maar ook die van de onbehandelde aandoening dienen meegenomen te worden in het behandelplan. [dit proefschrift]

3. Inconsistente onderzoeksresultaten vormen geen excuus voor drugsgebruik tijdens de zwangerschap. [dit proefschrift]

4. Digitale vragenlijsten zullen de traditionele methoden van dataverzameling in epidemiologisch onderzoek in de westerse wereld snel verdringen. [dit proefschrift]

5. Alleen góed meten is weten. [dit proefschrift]

6. Het doel van etiologisch onderzoek is niet het detecteren van associaties, maar het identificeren van mogelijkheden voor preventie.

7. Wanneer het *restricted choice* principe in meer situaties toepasbaar zou zijn, wordt de medische praktijk een stuk minder complex.

8. Statistics: the only science that enables different experts using the same figures to draw different conclusions. [Evan Esar, 1899-1995]

9. Ondanks de introductie van de *Uniform Requirements for Manuscripts Submitted to Biomedical Journals* in 1979 is er nog steeds geen uniformiteit te ontdekken in de instructies voor auteurs.

10. De combinatie van een academische carrière, topsport èn een sociaal leven is praktisch onmogelijk.

Marleen van Gelder
Nijmegen, 6 juni 2012