RECREATIONAL DRUG USE: A MAJOR RISK FACTOR FOR GASTROSCHISIS?

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by

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Abbreviations

ACORN	A Classification of Residential Neighbourhoods
AFP	Alpha-Fetoprotein
ALSPAC	Avon Longitudinal Study of Pregnancy and Childhood
AWD	Abdominal wall defect
BCS	British Crime Survey
BDS	Slone Epidemiology Unit Birth Defects Study
BMI	Body mass index
CBDMP	California Birth Defects Monitoring Programme
CESDI	Confidential Enquiry into Stillbirths and Deaths in Infancy
CTG	Cardiotocograph
DHC	Dihydrocodeine (possible metabolite of heroin)
EDD	Expected date of delivery
EUROCAT	European Surveillance of Congenital Anomalies
GCMS	Gas Chromatography Mass Spectrometry
GHB	Gamma-hydroxybutyric acid
ICD 10	International Classification of Diseases – 10^{th} revision
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LMP	Last menstrual period
LREC	Local Research Ethics Committee
MDMA	3,4-Methylenedioxymethamphetamine (Ecstasy)
MREC	Multi-Centre Research Ethics Committee
MSαFP	Maternal serum alpha feto-protein
NorCAS	Northern Region Congenital Abnormality Survey
ONS	Office for National Statistics
OPCS	Office of Populations Censuses and Surveys
OR	Odds ratio
OTC	Over the counter
POD	Place of delivery
RIA	Radioimmunoassay
SGA	Small for gestational age
SIA	Small intestinal atresia
SIM	Selected ion mode

THC-COOH	Metabolite of cannabis
TPN	Total parenteral nutrition
Trent CAR	Trent Congenital Anomalies Register
UHL	University Hospitals of Leicester
VDU	Visual display unit
6-MAM	6-Monoacetylmorphine (metabolite of heroin)
95% CI	95% confidence interval

Terms

Binge drinking	Consumption of ≥6 units of alcohol on a single occasion
BMI	Body mass index=weight (kg) / height $(m)^2$
Class A or B drug	Heroin, cocaine, ecstasy, amphetamines, LSD etc.
Class C drug	Cannabis etc.
Recreational drug	a drug that is currently illegal in the UK.

Abstract

Aims: To test the hypothesis that the risk of gastroschisis is positively associated with the use of recreational drugs in the weeks immediately following conception and to validate data collected at maternal interview concerning recreational drug use during pregnancy using maternal hair analysis.

Methods: A matched case control study was carried out in three English health regions from January 2001 to August 2003. For each case, three live born controls were selected, matched by initial intended place of delivery, region and maternal age. Case note review and maternal interviews were used to collect information about risk factors for gastroschisis. Human hair was collected for analysis to validate interview data concerning recreational drug use. Conditional logistic regression analysis was used to estimate the mutually adjusted odds ratios for gastroschisis associated with any recreational drug use and class A or B drug use.

Results: Data was collected for 144 gastroschisis cases and 432 controls. Using maternal self-report data the adjusted odds ratio (OR) for gastroschisis associated with first trimester use of (i) any recreational drug was 2.20 (95%CI 1.13 to 4.26) and (ii) class A or B drugs was 3.59 (95%CI 1.36 to 9.47); adjusted for body mass index, marital status, aspirin use, home ownership, history of gynaecological infection or disease and smoking. Where possible, self-reported class A or B drug use was validated using LC-MS/MS hair analysis. As a proportion of the total of all identified class A or B drug users, 22.2% of gastroschisis case mothers and 27.3% of control mothers were identified from LC-MS/MS hair analysis.

Conclusions: There was a significantly increased risk of gastroschisis associated with the self-reported use of recreational drugs in early pregnancy, in particular class A or B drugs. Hair analysis can be used both to validate class A or B drug use and to identify additional class A or B drug users.

Introduction: Part One – Gastroschisis

1.1 Background

The surgeon Ambroise Paré first described the abdominal wall defect omphalocele in his book "The Workes" published in 1634 (Pare, 1634). A century later Calder (1733) described two children with 'preternatural conformation of the guts' in a book of medical essays and observations. This was the first reference specifically made to a gastroschisis. However, it was not until 1953 that a clear distinction was made between gastroschisis, exomphalos and other abdominal wall defects (Moore and Stokes, 1953). Three separate abdominal wall defects were described in these case reports: Omphalocele (umbilical cord anomaly); Intussusception of ileum through persistent omphalomesenteric duct (omphalomesenteric duct anomaly); and Gastroschisis (extraumbilical abdominal wall anomaly). Separate codes for categories of abdominal wall defect were, nevertheless, not available in the International Classification of Diseases (ICD) until the 10th revision published in 1992 (see Figure 1.1). In previous ICD versions all abdominal wall defects had been grouped together in the category 'anomalies of abdominal wall' thus creating problems for epidemiological studies of the trends of each individual anomaly.

Figure 1.1: ICD 10 Codes for Abdominal Wall Defects

ICD10 Code	Anomaly
Q79.0	Congenital diaphragmatic hernia
Q79.1	Other congenital malformations of diaphragm
Q79.2	Exomphalos / omphalocele
Q79.3	Gastroschisis
Q79.4	Prune Belly Syndrome
Q79.5	Other congenital malformations of abdominal wall
Q79.6	Ehlers-Danlos Syndrome
Q79.7	
Q79.8	Other congenital malformations of the musculo-skeletal system
Q79.9	Congenital malformation of the musculo-skeletal system, unspecified

A relationship between abdominal wall defects and limb defects has been observed and described in the literature for many years. Suggestions have been made of a possible shared aetiology between these defects based on the rupture of the amnion in early development and the subsequent secondary effects (Pagon et al. 1979). This condition has been categorised as the limb body wall complex (Van Allen et al. 1987) to distinguish it from gastroschisis and exomphalos. Other authors have proposed a further classification of body wall defects (Hartwig et al. 1989) based on the abnormal development of the umbilical cord and the abdominal wall due to a malfunction in the ectodermal placodes which suggest that the defect results from embryonic dysplasia.

The limb body wall complex is a separate entity from gastroschisis. It is an evisceration of the thoracic and/or abdominal organs associated with other anomalies which may include limb deficiencies. This group of anomalies includes body stalk anomalies, which are severe defects of the abdominal wall with a very small or absent umbilical cord or a continuation of the placenta. Other body wall defects can be divided into those which are or are not produced by amniotic bands. (Martinez-Frias et al. 2000).

This thesis will focus on the abdominal wall defect gastroschisis.

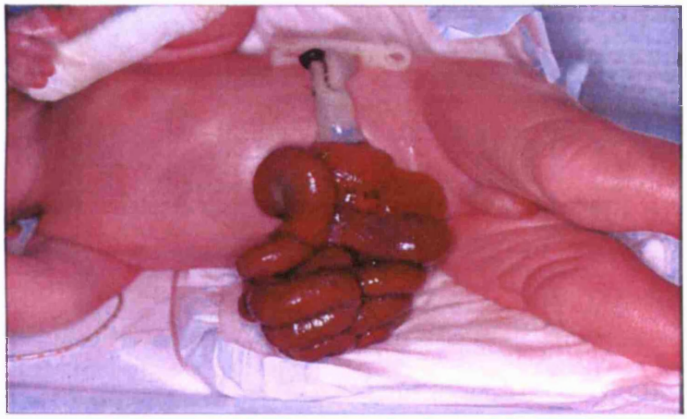
1.2 Gastroschisis

1.2.1 Description of the anomaly

Gastroschisis is an anomaly involving all layers of the anterior abdominal wall (ICD 10 code: Q79.3). The term gastroschisis is misleading as its literal meaning is a "split or open belly" (from the Greek 'gastro' belly and 'schisis' schism or split) whereas it is the abdominal wall and not the stomach which is open. It is a para-umbilical defect of approximately two to four centimetres in size, usually occurring to the right of the umbilical cord and associated with evisceration of the intestine.

In this condition a number of other organs are also often found to be extra-abdominal, including the stomach, bladder and gonads, the liver, however, does not herniate through the defect. The eviscerated intestines and organs are not enclosed in a sac and are therefore exposed to the amniotic fluid in the uterus during pregnancy (Figure 1.2). As a result the intestines may become shortened, thickened and dilated and are often matted together with adhesions (Kilby et al. 1998); (Moore and Persaud, 1998); (World Health Organisation, 1992); (Buyse, 1990).

Figure 1.2: Photo of a baby with a gastroschisis



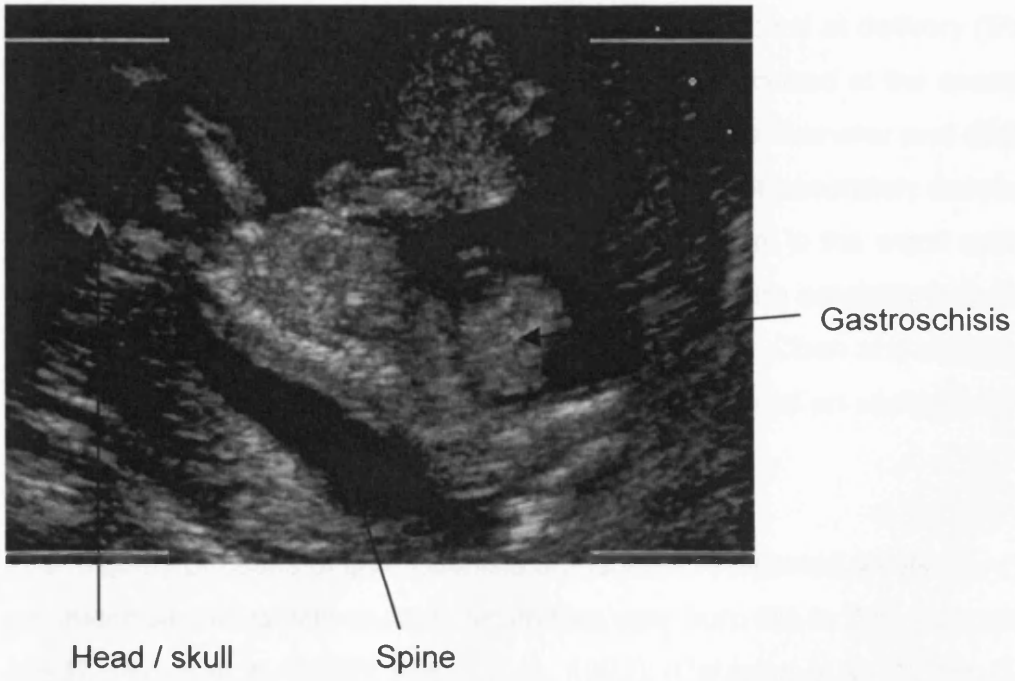
Source: S Marven, Sheffield Children's Hospital, with permission.

1.2.2 Prenatal Diagnosis of Gastroschisis

Following the implementation of routine antenatal screening for neural tube defects using maternal serum alpha feto-protein (MS αFP) levels it became clear that other fetal anomalies, including abdominal wall defects, could also be identified using this test (Leschot and Treffers, 1975). This has subsequently been confirmed for gastroschisis as the majority of pregnancies with a fetus affected by gastroschisis have raised maternal serum alpha-fetoprotein levels, with values of up to 4 or 5 times the median being common (Morrow et al. 1993), (Nielsen et al. 1985), (Redford et al. 1985). Gastroschisis cases are usually diagnosed at mid-

trimester detailed ultrasound anomaly scan by visualising the loops of bowel that herniate through the anterior abdominal wall into the amniotic fluid (see Figure 1.3). The first reported case of prenatally diagnosed gastroschisis was by Giulian and Alvear in 1978 (Giulian and Alvear, 1978). The criteria for ultrasound

Figure 1.3: Ultrasound picture of a baby with a gastroschisis



Source: N Marlow, Queen's Medical Centre, with permission.

diagnosis of abdominal wall defects are well reported (Redford et al. 1985), (Nielsen et al. 1985). The three most important features for distinguishing between different abdominal wall defects are the presence or absence of a sac, the position of the umbilical vessels entering the abdomen and which abdominal organs are involved. In gastroschisis cases there is no sac over the herniated fetal organs; the umbilical vessels enter the abdominal wall adjacent to the defect; the liver is always normally situated and in the majority of cases only the large or small bowel are involved (although the stomach, bladder and gonads may also be involved). Published prenatal detection rates over the past decade have varied from 66% (Chen et al. 1996) to as high as 95% (Fisher et al. 1996), (Forrester et al. 1998), (Dillon and Renwick, 1995), (Roberts and Burge, 1990). In 1995 to 1996

the prenatal detection rate for gastroschisis in the West Midlands health region was 97.5% (39 out of 40 cases with 35 (87.5%) of the cases being detected before 24 weeks gestation) (Kilby et al. 1998).

Following prenatal detection of a fetus with gastroschisis, serial ultrasonography is carried out to monitor fetal growth and to assess the condition of the intestines. Clinical evidence has suggested that the long-term prognosis for gastroschisis cases is mainly dependent upon the condition of the bowel at delivery (Stringel and Filler, 1979). However, a number of studies have looked at the association between the prenatal sonographic measurement of the diameter and dilatation of the small bowel and concluded that sonography cannot accurately determine the amount of bowel damage (Babcook et al. 1994), except in the worst cases (Pryde et al. 1994). Ultrasonography is also used to monitor the amniotic fluid volume as gastroschisis may be associated with oligohydramnios. Chen and colleagues (Chen et al. 1996) found that half of their case series had an associated oligohydramnios.

The majority of cases of gastroschisis are isolated. Reported levels of gastroschisis cases with multiple anomalies vary from 6% to 25% (Morrow et al. 1993); (Fisher et al. 1996); (Yang et al. 1992); (Calzolari et al. 1995); (Torfs et al. 1990); (Byron-Scott et al. 1998); (Calzolari et al. 1993); (Roberts and Burge, 1990). These anomalies range from secondary associations such as intestinal atresia and cryptorchidism to unrelated anomalies including skeletal anomalies, encephalocele and in rare cases aneuploidy or amniotic band sequence. Associations between a non-familial form of arthrogryposis, (a condition also known as amyoplasia where there are multiple congenital contractures) (Reid et al. 1986) and gastroschisis have led to the suggestion of a shared aetiology of vascular compromise. Links between gastroschisis and amelia (absence of limb(s)) (Rosano et al. 2000) have also been described. Conditions secondary to gastroschisis include urinary tract obstruction (Reiss et al. 2000) and cryptorchidism (Kaplan et al. 1986). In abdominal wall defects the intra-abdominal pressure is significantly lowered. This is postulated to affect the process of testicular descent increasing the incidence of cryptorchidism in affected male infants (Kaplan et al. 1986).

Although the prenatal identification of a gastroschisis has little effect upon the outcome for an individual case (Roberts and Burge, 1990); (Gabriel et al. 1992) it does allow time for parental counselling concerning the perinatal management of and the prognosis for the infants. Parents also have time to prepare for any medical or surgical requirements of the infant following delivery. Information derived from ultrasonography and the detection of any associated conditions help determine the likely prognosis for the infant.

1.2.3 Delivery and perinatal management

There has been controversy over the mode, timing and place of delivery for cases of gastroschisis. Many observational studies have looked at the effect of mode of delivery upon the outcome of gastroschisis cases (Sakala et al. 1993); (Adra et al. 1996); (Bethel et al. 1989); (Blakelock et al. 1997); (Novotny et al. 1993); (Quirk et al. 1996); (How et al. 2000); (Lewis et al. 1990); (Rinehart et al. 1999); (Snyder, 1999); (Moretti et al. 1990); (Tawil and Gillam, 1995). Although some have suggested that elective caesarean section at or before the onset of labour may benefit a gastroschisis fetus when compared with undergoing labour and vaginal delivery (Sakala et al. 1993) the majority of studies have found that delivery by caesarean section confers no benefit in terms of mortality and morbidity (Adra et al. 1996); (Bethel et al. 1989); (Blakelock et al. 1997); (Novotny et al. 1993); (Quirk et al. 1996); (How et al. 2000); (Lewis et al. 1990); (Rinehart et al. 1999); (Snyder, 1999); (Moretti et al. 1990); (Tawil and Gillam, 1995). However, due to the rarity of gastroschisis, most of these studies were small, retrospective and observational. Definitive data are not available from randomised controlled trials from which conclusions could be drawn. In addition, individual studies posed the question in different ways leading to conclusions such as Moretti et al who found that "vaginal delivery of infants with abdominal wall defects does not adversely affect infant outcome" (Moretti et al. 1990); Rinehart et al who conclude that "outcomes of infants with isolated gastroschisis were not significantly affected by method or site of delivery" (Rinehart et al. 1999); and Simmons et al who determine that "early elective caesarean section has no benefit

for cases of gastroschisis" (Simmons and Georgeson, 1996). Similarly, there are differing views about the best place to deliver a known case of gastroschisis. A number of studies have suggested that regional centres are the optimal place for delivery (Quirk et al. 1996), (Dykes, 1996) as neonatal, medical, surgical and anaesthetic expertise is immediately available, however, others have found that place of delivery has no effect upon the outcome of the infant with gastroschisis (Stoodley et al. 1993), (Dillon and Renwick, 1995), (Nicholls et al. 1993) provided that both optimal neonatal resuscitation and postnatal transfer to a surgical centre are available and the distance is not too great (Nicholls et al. 1993).

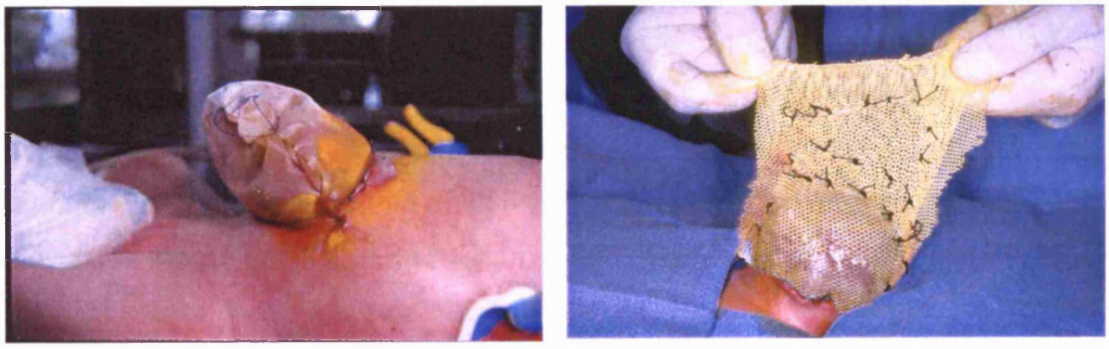
The median gestational age at delivery of gastroschisis cases is reported to be around 37 weeks (Tan et al. 1996), (Dillon and Renwick, 1995), (Adra et al. 1996), (Torfs et al. 1994). Policies concerning the optimal timing of delivery for gastroschisis cases vary. One reason for early intervention and delivery would be if bowel dilatation or wall thickening noted on antenatal ultrasound scan predicted the outcome for gastroschisis cases (Langer et al. 1993). However, such findings have been shown to be poor predictors of post-natal bowel complications (Babcook et al. 1994) and as preterm delivery has its own associated risks it is recommended only if there is convincing evidence that it will improve the outcome for gastroschisis cases. Burge et al (1997) suggest that gastroschisis cases are at significant risk of fetal distress that could indicate either an increased risk of intra-uterine death or adverse neurological outcome. As such, repeated cardiotocograph (CTG) monitoring in the third trimester of pregnancy could ensure appropriate preterm intervention in these high-risk cases. Other studies have found that although gestational age at delivery is not correlated with survival in gastroschisis infants, those infants that are preterm have increased problems of sepsis (Snyder, 1999) and that term delivery (with primary closure of the defect) is likely to minimise the morbidity of these infants (Blakelock et al. 1997).

1.2.4 Post-natal Management and Outcome

Following delivery and any required resuscitation of infants with gastroschisis the external bowel is wrapped in a "bowel bag" or cling film to minimise heat and fluid loss (Driver et al. 2001); (Tawil and Gillam, 1995); (Kilby et al. 1998). The infant is then stabilised and transferred to the nearest appropriate theatre for surgical repair. Although immediate closure of the defect has always been considered to be the optimal surgical practice Driver et al (2001) found that there was no correlation between time to closure and outcome (median time to closure: 4 hours, ranging from 0.5 to 17 hours), thus allowing time for adequate resuscitation without having any detrimental effect upon outcome.

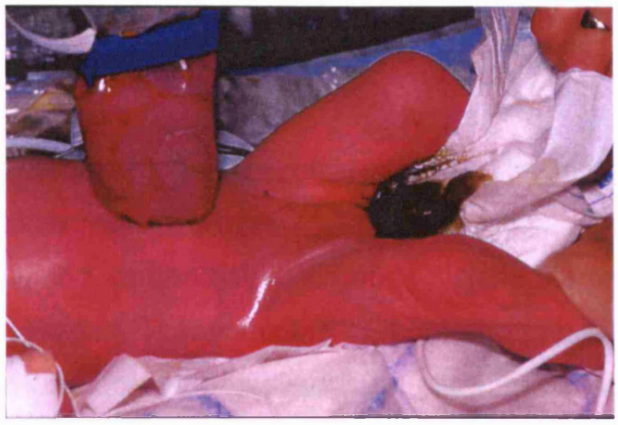
Primary closure of the defect is usually attempted unless there is likely to be a problem with skin closure or venous drainage (Tawil and Gillam, 1995), (Driver et al. 2001). In these cases a staged repair is carried out involving the construction of a "silo" pouch. Silos can be constructed out of a variety of materials, see Figures 1.4 and 1.5 (Lee et al. 1997); (Minkes et al. 2000); (Schlatter, 2003), (Sandler et al. 2004), (Bhatnagar et al. 2001)).

Figure 1.4: Conventional silo: Dacron reinforced silicone



Source: S Marven, Sheffield Children's Hospital, with permission.

Figure 1.5: Preformed Silo



Source: S Marven, Sheffield Children's Hospital, with permission.

The silo is then gradually reduced until reduction is achieved and final abdominal closure is carried out in theatre. Some studies have suggested that silo repair cases have an increased complication rate and longer length of hospital stay than those undergoing primary closure (Luck and Scrutton, 1997); (Nicholls et al. 1993); (Blakelock et al. 1997) however Tawil et al (1995) found similar outcomes in both groups. More recently some units have used a minimal intervention method of manual reduction of the defect without anaesthesia or sedation in an incubator on the paediatric surgical unit (Bianchi and Dickson, 1998); (Kimble et al. 2001), (Jona, 2003), with apparently similar outcomes to primary closure in theatre. However, a recent Cochran review has suggested that as there is an urgent need for a randomised controlled trial to compare ward reduction versus reduction under general anaesthesia in infants with gastroschisis to provide evidence to either support or refute this method of treatment (Davies et al. 2002). Total parenteral nutrition (TPN) is commenced once the infant is stabilised after primary or final closure of the defect (Tawil and Gillam, 1995); (Driver et al. 2001) and continued until full enteral feeds are established.

Serious complications may arise after gastroschisis repair including necrotising enterocolitis, sepsis, short-bowel syndrome, persistent small-bowel dysfunction and cholestatic jaundice (Novotny et al. 1993), (Ramsden et al. 1997) (Thakur et

al. 2002) which may lead to further requirements for operative procedures in these infants. In particular infants with short gut/resection and bowel atresia require an extended period of TPN and hospitalisation and experience significant morbidity and complications (Tawil and Gillam, 1995); (Cusick et al. 1997) thus requiring neonatal intensive care facilities.

Long-term survival data for gastroschisis cases are limited. Many of the data originate from either radiology departments, who are interested in the accuracy of prognostic ultrasound, or paediatric surgeons, whose starting point is admission to a unit for surgical repair. These studies have tended to be small and limited to the cases treated by one unit. Neonatal survival following the antenatal detection of gastroschisis has been reported to be as high as 92% to 94% (Babcook et al. 1994), (Brun et al. 1996). Follow-up studies have been carried out over a variety of time periods. Mabogunje and colleagues (1984) looked at trends in the survival of gastroschisis cases (following admission to a paediatric surgical unit) to over one year of age, over four quinquennia from 1960-65 to 1975-80 and found a dramatic increase from 18% to 91%. Other, more recent, series have found survival rates to discharge from hospital (Ramsden et al. 1997) and to one year of age in excess of 90% (Stringer and Mason, 1997), (Tawil and Gillam, 1995). Studies of longer term survival following paediatric surgery for gastroschisis have also reported very high rates: 88% to five years of age (Swartz et al. 1986); 90% in a follow-up of between 2 and 7 years (Cusick et al. 1997); 94% in a study where subjects were followed up for between 10 and 20 years by Tunell and colleagues (1995) in Oklahoma, USA; and, once again, 94% in a small UK study from Leeds (Davies and Stringer, 1997) where 35 gastroschisis survivors at one year of age were followed up for between 12 and 23 years. However, Cusick et al (1977) suggest that there is a significantly higher risk of death in gastroschisis cases with small bowel atresia or stenosis and that because most follow-up series are restricted to unit level, this problem is both concealed and limits experience in the management of these cases.

Long term studies of the morbidity of gastroschisis survivors have reported good health status and normal growth in the majority of cases (Davies and Stringer, 1997), (Tunell et al. 1995); (Swartz et al. 1986). Complications such as adhesive

bowel obstruction occur in some cases (Davies and Stringer, 1997); (Wilkins and Spitz, 1986) and children with concomitant bowel atresia are at an increased risk of long term bowel problems and abdominal complaints (Swartz et al. 1986). Long term survivors with this condition have reported distress as a child due to their lack of an umbilicus (a resultant of poor surgery) (Davies and Stringer, 1997); (Tunell et al. 1995) leading to problems with sports and other social activities (Tunell et al. 1995).

Congenital Anomaly Registry data can provide information concerning the outcome following delivery for population based cohorts of antenatally and post-natally diagnosed cases. For example, data from the Northern Region Fetal Abnormality Survey, excluding first trimester losses, indicated that over the period 1988 to 1992 86% of gastroschisis cases were live born (Dillon and Renwick, 1995), (Fisher et al. 1996). In contrast, data from the North Thames West Congenital Malformation Register from 1990 to 1993 showed that an unusually high proportion of gastroschisis cases had associated anomalies leading to a 23% rate of termination (Chitty and Iskaros, 1996).

1.2.5 Birth incidence or birth prevalence in relation to congenital anomalies.

In the area of birth defects there is an issue concerning the terminology to describe the extent of such conditions in a population. Should birth incidence or birth prevalence be used? Incidence is the number of new cases of a condition occurring divided by the time period over which such cases were followed. Given that gastroschisis occurs at some point between four and ten weeks of gestational age then the incidence rate is defined by the number of new cases of gastroschisis occurring, divided by the total number of all conceptions reaching four to ten weeks gestational age in a defined population of women for a defined period of time (Elwood and Elwood, 1980). Thus to calculate the incidence rate the fate of all conceptions reaching four to ten weeks gestational age must be known. Information about the rate of all early spontaneous losses at this early gestational age is not available. In addition, a precise definition of the denominator is required in the measurement of incidence (Hennekens and Buring, 1987) and this denominator should not include those who already have the disease or anomaly

as they are not at risk. Once again for early spontaneous abortions and terminations of pregnancy the presence or absence of an anomaly will rarely have been determined and therefore the incidence rate is not an appropriate instrument to use when estimating the frequency of gastroschisis and, indeed, birth defects in general.

The prevalence of a disease is the proportion of a defined population affected by a disease/condition at a specified point in time (Breslow and Day, 1992). When prevalence is defined over a period of time it is described as the period prevalence and can be used in the estimation of congenital anomaly rates (where the period tends to be given as the annual rate). Studies of congenital anomalies tend to quote the number of affected births as the numerator and the total number of live and stillbirths as the denominator. This denominator will be affected by the gestational age of the definition of a stillbirth in the specific country of the study (between 20 and 28 weeks gestational age). The prevalence ratio at birth of the congenital anomaly of interest is therefore the number of affected births divided by the total number of live and stillbirths. In studies where mid trimester loss data and termination of pregnancy data are collected for specific anomalies the denominator can be adjusted by including termination of pregnancy data for the population at risk but there are no estimates of the total number of mid-trimester losses available routinely for population data. The denominator used is therefore a slight underestimate but is the best possible denominator available.

For these reasons the measures of disease frequency referred to in this thesis are the total prevalence (ie. including terminations of pregnancy and late fetal losses) and the birth prevalence.

1.2.6 The Prevalence of Gastroschisis

A number of large scale epidemiological studies have reported an increase in the prevalence of gastroschisis over the past thirty years or so (Lindham, 1981), (Gierup et al. 1982), (Baird and MacDonald, 1981), (Martinez-Frias et al. 1984), (Morrow et al. 1993), (Tan et al. 1996), (Nichols et al. 1997), (Roeper et al. 1987), (Penman et al. 1998), (Forrester and Merz, 1999), (Rankin et al. 1999).

These studies encompass much of Europe, North America and Australia. An early report of the increased prevalence was from Sweden (Lindham, 1981) where there was a significant increase from 0.4 per 10,000 total births in 1965 to 1.11 per 10,000 total births in 1976, with a peak prevalence of 1.72 per 10,000 total births recorded in 1973.

Comparison of prevalence rates between areas is complicated by the inclusion criteria of each data collection system. There are systems that collect live birth anomaly data alone (Egenaes and Bjerkedal, 1982); (Martinez-Frias et al. 1984), some collect live and stillbirths (Bugge and Hauge, 1983) (Tan et al. 1996); (Roeper et al. 1987) while others collect all antenatally and postnatally diagnosed cases resulting in late fetal loss (≥ 20 weeks gestation) or termination of pregnancy, stillbirths and live births (EUROCAT, 2002). In addition international comparisons are complicated by the definition of stillborn infants that can vary from 20 to 28 weeks gestation, thereby affecting the size of the denominator and could, therefore, provide part of the explanation for the observed differences. A north-south gradient in prevalence across England and Wales has been suggested by Rankin and colleagues (1999) from the Northern Congenital Abnormality Survey. As this gradient has been observed for both gastroschisis and exomphalos cases differential reporting rates and the wide variation in regional uptake of terminations of pregnancy across the country could account, at least partially, for these differences (Tan et al. 1996).

Tan et al (1996) highlighted the problems of underreporting to the national notification system for congenital anomalies (NCAS) for England and Wales at the Office of National Statistics (ONS) and a recent study has shown that the level of underreporting may be as high as 40% (Boyd et al. 2005). The NCAS system of data collection is limited, in practice, to birth and early newborn notifications of anomalies. However, regardless of this problem an overall increase in the prevalence of gastroschisis was noted over the period 1987 to 1993. If underreporting remained constant (and there was no reason to believe that it did not at that time) then changes in birth prevalence can still be observed. However, since some regional registers (eg. Trent and CARIS in Wales) have now begun to

report directly to NCAS the level of underreporting has been substantially decreased and therefore this is no longer the case for the areas covered by those regional registers (Office for National Statistics, 2002).

Table 1.1 details the published prevalence rates for gastroschisis over the past three decades. The apparent increase in the prevalence of gastroschisis over time could be partly due to increased ascertainment and also a clearer definition of abdominal wall defects allowing clinicians to more accurately distinguish between cases of gastroschisis and exomphalos. In Denmark Bugge and Hauge (1983) reclassified all abdominal wall defects (using the Moore and Stokes classification, 1953), occurring in the national population between 1970 and 1979 and found that only 48% of the true gastroschisis cases had been correctly classified. Classification of these defects might therefore have had an impact on the apparent increase over time, although these problems are unlikely to have continued to increase overtime. Nichols et al (1997) showed a sharp rise in the occurrence of gastroschisis in mothers aged 15 to 19 years in Western Australia. The rate increased from 4.0 to 26.5 per 10,000 births over the period 1980 to 1993, with no similar increase in other age groups.

Table 1.1: Published prevalence rates per 10,000 births for gastroschisis, 1965 to 1997.

Location	Prevalence Gastroschisis per 10,000 births	Period of data collection	Author	Type of data
Sweden	0.65	1965-76	(Lindham, 1981)	National
Canada – BC	0.81	1969-78	(Baird and MacDonald, 1981)	Register
Norway	0.76	1967-79	(Egenaes and Bjerkedal, 1982)	National
USA – California	0.50	1968-77	(Roeper et al. 1987)	Birth & Death certificates
Northern Sweden	1.66	1970-74	(Gierup and Lundkvist, 1979)	Hospital
Denmark	1.41	1970-79	(Bugge and Hauge, 1983)	National
Spain	0.40	1976-81	(Martinez-Frias et al. 1984)	Register
Maryland USA	1.44	1980-87	(Yang et al. 1992)	Hospital
Western Scotland	1.86	1983-89	(Morrow et al. 1993)	Hospital
Italy	0.60	1984-89	(Calzolari et al. 1993)	Register
Europe	0.94	1980-90	(Calzolari et al. 1995)	Registers
Western Australia	1.80	1980-93	(Nichols et al. 1997)	Register
Southern & Western Australia	1.65	1980-90	(Byron-Scott et al. 1998)	Register
China	1.60	1986-87	(Zhu et al. 1996)	Register
England & Wales	1.11	1987-93	(Tan et al. 1996)	Office for National Statistics
North West Thames	1.60	1990-93	(Chitty and Iskaros, 1996)	Register
Scotland	1.89	1988-95	(Chalmers et al. 1997)	Information & Statistics Division
Scotland	1.60	1988-93	(Chalmers et al. 1997)	Information & Statistics Division
South West of England	1.60 to 4.40	1987-95	(Penman et al. 1998)	Hospital records
Hawaii	3.01	1986-97	(Forrester and Merz, 1999)	Register
Northern England	2.98	1986-96	(Rankin et al. 1999)	Register
Japan	0.13 0.27 0.37 0.46 0.47	1975-80 1981-85 1986-90 1991-95 1996-97	(Suita et al. 2000)	Special survey

The most recently available data from the larger EUROCAT registries (with an annual number of births of at least 20,000) is presented in Table 1.2 for the triennium 2000 to 2002. These registries have a standardised method of data collection and are therefore directly comparable (EUROCAT, 2002). The prevalence of gastroschisis varies from 0.25 per 10,000 births in Tuscany to 4.21 per 10,000 births in Paris and 4.32 per 10,000 births in the UK region of Trent. An increasing prevalence for gastroschisis can be seen from Southern to Northern Europe.

Table 1.2: Gastroschisis (total prevalence per 10,000 births) from EUROCAT registries with annual births of at least 20,000 over the period 2000 to 2002.

EUROCAT Registry		Total Births 2000 to 2002	Gastroschisis prevalence per 10,000 total births
Paris	(France)	116,364	4.21
Dublin	(Ireland)	65,968	2.88
Campania	(Italy)	162,593	0.55
Emilia Romagna	(Italy)	79,069	1.01
North East Italy		162,328	0.62
Tuscany	(Italy)	79,590	0.25
North Netherlands		61,380	0.98
Wielkopolska	(Poland)	103,367	1.93
CARIS	(Wales)	92,589	3.67
North Thames	(UK)	137,856	1.67
Trent	(UK)	164,444	4.32
Wessex	(UK)	77,674	2.96

1.2.7 Aetiology of Gastroschisis

The aetiology of gastroschisis has been the subject of considerable debate for many years. Theories have included the following: that a teratogenic event during early development could result in a defect in the differentiation of the embryonic mesenchyme leading to the resorption of the somatopleure, creating the para-umbilical defect (Duhamel, 1963); that a similar event could lead to the dysplasia of the mesoderm of the umbilical ring followed by a later disruption (Vermeij-Keers et al. 1996), or that the membrane covering the umbilical cord hernia ruptures in utero thus forming a gastroschisis (Shaw, 1975). More recent theories have focused on a vascular pathogenesis for gastroschisis. These suggest that gastroschisis is either due to a vascular accident which causes occlusion of the right omphalomesenteric artery, resulting in necrosis at the base of the umbilical cord, creating an opening through which the intestines can eviscerate (Hoyme et al. 1981); or to the premature atrophy or abnormal persistence of the right umbilical vein leading to mesenchymal damage and failure of the epidermis to differentiate at that site (deVries, 1980)

The timing of the formation of gastroschisis is also the subject of some debate. Most concur with the deVries and Hoyme (deVries, 1980), (Hoyme et al. 1981) theories in that the postulated timing of the development of this defect occurs sometime between four to five weeks gestation when the right umbilical vein involutes and the left omphalomesenteric artery is ablated, and ten weeks gestation when the mid-gut is returned into the peritoneal cavity following a period of herniation into the extra-embryonic coelom. However, others suggest that the rupture of the umbilical membrane may occur either during the fifth to tenth weeks of gestation or nearer the time of birth (Shaw, 1975). This later or perinatal variant would appear to be both rare and of a totally separate aetiology.

Familial occurrence of gastroschisis supports a genetic aetiology at least for some cases. As reports of familial occurrence for gastroschisis in the literature are rare the risk of recurrence is thought to be small. Sibling recurrence of gastroschisis in unrelated families with a history of abdominal or umbilical hernia have been reported in one or two families (Salinas et al. 1979); (Ventruto et al. 1985).

Gastroschisis occurrence in a set of monozygotic twins has been reported with a separate report of a pair of siblings, one with gastroschisis and the other with exomphalos (Hershey et al. 1989). Data from the Californian Birth Defects Registry (1988 to 1990) reported that in six (4.7%) out of 127 extended pedigrees of families with a case of gastroschisis more than one relative was affected (Torfs and Curry, 1993). The sibling recurrence in these families was 3.5%; a higher recurrence risk than formerly thought. The first case of vertical transmission of gastroschisis from mother to son was reported in the literature in 1995 (Nelson and Toyama, 1995). The author postulated that this may be a new phenomenon because in the past gastroschisis cases did not survive to have offspring of their own.

In conclusion, the aetiology of gastroschisis is most likely multi-factorial and may well include a genetic component, at least for a small proportion of cases.

1.2.8 Suggested Risk Factors for Gastroschisis

Compelling evidence has been found from many studies of a link between young maternal age and an increased risk of gastroschisis (Nichols et al. 1997); (Haddow et al. 1993); (Tan et al. 1996); (Penman et al. 1998); (Goldbaum et al. 1990); (Werler et al. 1992a). Analysis of data from the Office of Population Censuses and Surveys (now ONS) national congenital anomalies notification system (NCAS) for England and Wales over the period 1987 to 1993 showed that the median age for mothers of gastroschisis cases was 21.0 years (inter-quartile range: 19 to 25). This was significantly lower than for the total births over this period: 26.8 years in 1987 and 28.0 years in 1993 (OPCS, 1987-1993). Data from the South West of England (Penman et al. 1998) from 1987 to 1995 demonstrated an eleven-fold increase in the risk associated with gastroschisis for mothers conceiving before the age of 20 years compared with those aged 20 years or more at conception. A similar risk was found in Western Australia (Nichols et al. 1997) where 15 to 19 year old mothers had ten times the prevalence of gastroschisis compared to mothers aged 25 to 29 years. This pattern was also found by Werler et al (1992a) in Boston, USA in the late 1970s and 1980s in a case-control surveillance programme of birth defects. In relation to mothers of 30 years or

more, mothers aged less than 20 years had a crude relative risk of 16.0 (95% ci, 8.1 to 30.0).

One theory for the increased risk of gastroschisis in young mothers is that there is an association between young maternal age and prenatal vascular disruptions (Lubinsky, 1997). Young maternal age is also linked with hydrancephaly, an anomaly where vascular disruption is also thought to be an aetiological factor. However, this is by no means conclusive and it could be that there is an, as yet unknown, protective effect related to age for older mothers.

There have been a number of case control studies designed to investigate factors that lead to an increased risk of gastroschisis. Two of the studies were of relatively poor quality as they were too small to have sufficient power to detect any as statistically significant clinically important risk factors. These studies collected information from the mothers of gastroschisis infants many years after their birth; and had response rates of less than 60% (Drongowski et al. 1991), (Eire et al. 1993). One further study from France, carried out over a twenty year period (1979 to 1998), looked at gastroschisis and exomphalos cases together, was limited to data from hospital notes and provided a limited, uncontrolled analysis (Stoll et al. 2001). A case control study carried out in Sweden in the mid 1970's failed to find any significant risk factors for gastroschisis (Lindham, 1983), whilst in the mid 1980's an American study, (Goldbaum et al. 1990) based on routinely collected data, confirmed young maternal age as a risk factor for gastroschisis with smoking in pregnancy and seasonality also remaining statistically significant following multivariate analysis. Haddow and colleagues (1993) studied these factors in more detail and found no evidence for seasonality as a risk factor but confirmed both young maternal age and smoking during pregnancy as risk factors, the latter on combining data from previous studies (Goldbaum et al. 1990), (Werler et al. 1992a). A Spanish study (Martinez-Frias et al. 1997) carried out over a twenty year period (1976-1996) found a three-fold increased risk of gastroschisis in women who had taken salicylates in their first trimester. However, the data collection concerning salicylate use was inadequate for detailed analysis. An association between maternal employment in the printing industry and gastroschisis has been suggested by a group from Atlanta (Erickson et al. 1978).

This finding, was however, based on congenital anomaly cases alone without comparative data for the mothers of control babies and has not been replicated in other studies. A pan-European case control study (EUROHAZCON) looked at the risk of congenital anomalies associated with residing near hazardous-waste landfill sites (Dolk et al. 1998). The associated risk of gastroschisis for living near a hazardous-waste landfill site was found to be three-fold with borderline statistical significance. However, as this finding was based on just 13 cases of gastroschisis the authors stressed the need for caution when interpreting the results and recommend further research. Further evidence of a link between gastroschisis and landfill sites was produced from an ecological study in Wales (Fielder et al. 2000). Once again, however, this evidence was not conclusive and was based on a cluster of only four gastroschisis cases.

The two major series of case control studies investigating the aetiology of gastroschisis have been conducted in the USA. The first of these was by Claudine Torfs and colleagues in California, (Torfs et al. 1994,1996, 1998); (Lam et al. 1999). This study used all singleton births notified to the California Birth Defects Monitoring Programme (CBDMP) over the period March 1988 and August 1990, clearly classified as having a gastroschisis by a clinical geneticist. Two controls were selected for each case from a random selection of all Californian births, matched to within one year of the case mother's age and for racial group. All subjects were interviewed at home within three to six months of delivery, with interviewers blinded to the study hypothesis. Out of the original 157 mothers of infants with gastroschisis 110 were used in the analysis. Of the 37 mothers not used in the analysis 14 (8.9%) were refusals, 16 (10.2%) were lost to follow-up and 17 (10.8%) were excluded due to inadequate racial matching. Levels of refusal and loss to follow-up were slightly higher in the control group: 10.9% and 11.6%, respectively. Initial analysis from this study concentrated on socio-demographic, pregnancy and lifestyle risk factors including maternal educational level, family income, details of mothers childhood, time interval between menarche and the first or index pregnancy and recreational drug use (including tobacco and alcohol) both in the immediate pre-conceptional trimester and the first trimester of pregnancy. Although univariate analysis showed a significant association between many of the factors and gastroschisis the conclusion of the study following

conditional logistic regression analysis was that "socially disadvantaged women with a history of substance use were at highest risk of a child with a gastroschisis" (Torfs et al. 1994). Marijuana was the only recreational drug to remain significant in the regression model although the association between cocaine use and gastroschisis remained strong but was not statistically significant. Cocaine use can result in the activation of the adrenergic systems that can potentially impair the circulation leading to hypertension, tachycardia and vasoconstriction (Volpe, 1992) and a possible link between this vasoactive drug and gastroschisis has been hypothesised by Drongowski et al (1991). However the study by Drongowski et al (as previously mentioned) was methodologically flawed with only small numbers, the collection of data from maternal interviews up to five years after the delivery of the birth and no multivariate analysis.

Under-ascertainment of recreational drug exposure can be a major problem in studies collecting data from maternal interview and notes reviews (Torfs et al. 1994), (Colon et al. 2001), (Elman et al. 2000). There may also be a differential reporting, leading to information bias between responders and non-responders (Embree and Whitehead, 1993) especially when comparing the mothers of malformed and normal infants (Stott, 1958), (Lieff et al. 1999a), (Swan et al. 1992), (Werler et al. 1989). Efforts were made in this CBDMP study (Torfs et al. 1994) to account for some of these problems, including the collection of information about father's recreational drug use as it was felt that women were more likely to be truthful about their partner's use than their own, but no direct method of validation of interviewed data was carried out.

A secondary analysis of this CBDMP study was carried out to investigate the risk of gastroschisis associated with maternal medications and environmental exposures (Torfs et al. 1996). Conditional logistic regression analysis adjusting for the factors found previously (Torfs et al. 1994) indicated that most of the significant associations in this study were for vasoactive substances which would support a vascular hypothesis for the aetiology of gastroschisis. These fell into three groups of compounds where exposure was associated with gastroschisis: cyclooxygenase inhibitors, aspirin and ibuprofen; organic solvents; and two decongestants, pseudoephedrine and phenylpropanolamine. First trimester

exposure to X-rays was also associated with an increased risk of gastroschisis and it was postulated that X-ray exposure might compromise the abdominal wall of the fetus in some way.

A further analysis to test a nutritional hypothesis suggested an association between gastroschisis and poor maternal diet, in particular, low levels of glutathione and α-carotene and high levels of nitrosamine (Torfs et al. 1998). Data about nutrient intake, during the three months prior to conception, was collected using a self-report food frequency questionnaire completed within three to six months of the delivery of their infant. This time point was chosen as mothers were thought to be more likely to remember their usual diet than their diet during pregnancy, thus minimising recall bias and highlighting long term deficiencies in their diet. Differential recall between cases and controls was also thought to be unlikely. Early problems with the study design were addressed but led to a poor response to this questionnaire in case mothers. Following further exclusions the final analysis for this study was carried out using 55 (50%) cases mothers and 182 (83%) control mothers. Responders and non-responders were, however, found to be very similar in terms of socio-demographic factors, vitamin use, food bingeing, smoking and body mass index. As this was an exploratory study, analysis of 38 separate nutrient variables was carried out in order to generate hypotheses concerning any suggested links between nutrient intake and gastroschisis. Three nutrient factors emerged from this analysis, namely low levels of glutathione and α-carotene and high levels of nitrosamine. These were subsequently investigated in a multivariable model adjusting for the risk factors from previous studies (Torfs et al. 1994), (Torfs et al. 1996) in order to detect any confounders or interactions between variables. Recreational drug use (included in the model as a binary variable) and aspirin or ibuprofen use were the only previous factors to remain in the model and neither achieved statistical significance leaving a somewhat confused picture. The results from this analysis are described as tentative and the authors conclude that "teenage children or young adults who are at a social disadvantage or have poor nutrition are more likely to have an infant with a gastroschisis". This, however, needs confirmation from further studies.

In order to further investigate nutritional hypotheses in this study an analysis was carried out to determine if underweight women are at a higher risk of having an infant with a gastroschisis (Lam et al. 1999). Using the mother's pre-pregnancy body mass index (kg/m^2) as a continuous variable, adjusting for the variables found to be of significance in the previous analyses, it was found that the risk of gastroschisis increased by approximately 11% with every unit decrease in the body mass index. This finding added weight to the group's "poor nutrition" theory (Torfs et al. 1998).

The other major case control studies established to investigate the risk factors associated with gastroschisis are from Werler and colleagues in Boston (Werler et al. 1992a, 1992b, 2002, 2003). In the first of these studies, cases of gastroschisis were identified from the Slone Epidemiology Unit Birth Defects Study (BDS) for the period 1976 to 1990. This is a hospital based case control surveillance system (Werler et al. 1992a). Home interviews were carried out by a nurse interviewer, within 6 months of delivery, using a structured questionnaire to collect information about socio-demographic, reproductive and environmental factors including risk behaviours and medication use. Prior to 1983 details concerning smoking patterns and alcohol consumption were not collected. All 76 cases of gastroschisis notified to the BDS were included in the study, although due to the addition of exposure data items at different time points through the study the number of cases interviewed for exposure data was between 49 (64%) and 60 (78%). A one in four random sample of the remaining infants with major structural defects on the BDS were identified as controls. Selection of the controls was carried out following the exclusion of infants with chromosomal anomalies, due to their strong association with maternal age, and infants with any other abdominal wall and associated defects, to avoid any potential misclassification. In total, 2,581 control infants were selected of whom between 1726 (67%) and 2152 (83%) were interviewed to collect exposure data.

The authors gave consideration to a number of study limitations. Analysis of the study was carried out both with and without cases of gastroschisis that were associated with other anomalies to allow for the possibility of aetiological heterogeneity. This analysis did not change the conclusions of the study.

Procedures for the recruitment of cases into the study and the collection of information were considered for the possibility of selection bias as the study was neither population based nor were all gastroschisis cases approached for inclusion. The authors indicated that they felt such bias was minimal as the potential risk factors being evaluated were unlikely to be affected by recruitment procedures. Sub-analyses of anomaly groups in the controls were carried out to check for any reporting bias of exposures in case knowledge of exposure/anomaly associations were known. Issues concerning differential reporting of exposures between cases and controls were addressed by the use of controls with anomalies. However, recall in all groups was still a problem as subjects were asked to recall exposure from one year ago and there was no method of validating interviewed data. Observer bias was minimised by concealing the study hypotheses.

A primary analysis of the demographic, reproductive, medical and environmental factors associated with gastroschisis confirmed the known association with young maternal age and identified a number of potential risk factors in the univariate analysis. Following adjustment for age, all factors, with the exception of the consumption of five or more drinks of alcohol at any one time, were not significant. The authors concluded that some suggested risk factors for gastroschisis, for example, reproductive factors and cigarette smoking, could be attributable to age differences in cases and controls and they suggested that the alcohol consumption finding was only tentative (Werler et al. 1992a).

In a subsequent logistic regression analysis of first trimester maternal medication use and the associated risk of gastroschisis, a three-fold increased risk was found for those mothers who used pseudoephedrine (a constituent of many cold remedies) in the first trimester of pregnancy (Werler et al. 1992b). Other vasoactive drugs including salicylates (aspirin), acetaminophen (paracetamol), and phenylpropanolamine showed elevated relative risks but were non-significant following adjustment for maternal age, years of education, parity, alcohol consumption, influenza in the first trimester, other medications, interview period and study centre.

A further case control study was established to measure the risk of gastroschisis and small intestinal atresia associated with cold and antipyretic/analgesic medications in early pregnancy (Werler et al. 2002). The study was conducted between June 1995 and March 1999 in 15 cities across the USA and Canada during which time 206 cases of gastroschisis were recruited. This represented a 79% response rate from eligible cases. Two groups of hospital controls were selected: infants with structural anomalies other than gastroschisis, small intestinal atresia or other gastrointestinal anomalies (382 recruited: 73% response rate), and infants without anomalies (416 recruited: 68% response rate) with medical conditions that required hospital admission (for example, infections, prematurity, gastrointestinal problems and seizures). These infants without anomalies were therefore plausibly more likely to be on antipyretic and analgesic medications. In this study, telephone interviews were carried out with mothers within 6 months of delivery, and meticulous attention was paid to the detailed collection of illnesses and medication use in early pregnancy. Similar issues of bias were present in this study as in the previous study although one additional problem was with the recruitment of some controls. Recruitment was prevented because either they were not contactable or their physician refused permission for the family to be approached resulting in a larger proportion of the ascertained controls being ineligible for the study in comparison to the cases. Recall bias was reduced in this study by providing a booklet containing photographs of medications and their packaging to assist the memory of the subjects.

The conclusions from this study were that there was a statistically significant threefold increased risk of gastroschisis with use of aspirin in early pregnancy (OR 3.2; 95%CI 1.4 to 7.1). First trimester use of acetaminophen (paracetamol) was associated with a 50% increased risk of gastroschisis (OR 1.5; 95%CI, 1.1 to 2.2) whereas ibuprofen showed no statistically significant excess risk. Although taking pseudoephedrine alone in early pregnancy showed no increased risk of gastroschisis, pseudoephedrine in combination with acetaminophen during this period showed a significant excess risk with an odds ratio of 4.2 (95% CI, 1.9 to 9.2).

The next stage of this study (Werler et al. 2003) was to investigate the hypothesis that the use of vaso-constrictive drugs in early pregnancy increases the risk of gastroschisis and small intestinal atresia. In this analysis the researchers looked at the singular use of both medicinal and recreational vaso-constrictive drugs including pseudoephedrine, phenylpropanolamine, ephedrine, MDMA (ecstasy), amphetamines, cocaine and crack and their combined use with cigarette smoking. The authors recognised the problems with the 'truthful' provision of information about the use of recreational drugs and they therefore considered that any reported recreational drug use was likely to be an underestimate of the true prevalence in the study population. Potential confounding factors such as other recreational drugs, alcohol consumption and use of other medication for conditions similar to those requiring decongestants were also considered.

The main findings of this study, following conditional logistic regression analysis, were that whilst the singular use of vaso-constrictive drugs or smoking in early pregnancy were associated with an increased risk of gastroschisis (adjusted OR for vaso-constrictive drug use 1.7; 95%CI, 1.0 to 2.8), their combined use had a multiplicative effect on this risk (adjusted OR 3.1; 95%CI, 1.7 to 5.7). They concluded, however, that a true estimate of this risk would be difficult to obtain without the use of a validation technique for the data concerning drug use during early pregnancy.

1.3 Summary

Gastroschisis is a rare anomaly that has shown an increasing prevalence over the past thirty years or so. The majority of gastroschisis cases are prenatally diagnosed thus allowing time for parents for counselling and preparation for the birth. Although survival rates from this anomaly are high, gastroschisis cases require paediatric surgery and neonatal intensive care. The aetiology of gastroschisis is most likely multi-factorial and most theories focus on a vascular pathogenesis early in the first trimester. A strong association between young maternal age and an increased risk of gastroschisis has been shown. A variety of

risk factors for gastroschisis have been suggested including social disadvantage, smoking, poor diet, environmental factors and the use of vaso-active drugs, both medications and recreational drugs. However, estimates of the risk associated with recreational drug use have been limited, as data collection concerning first trimester recreational drug use has not been validated.

Introduction Part Two – Epidemiological Methodology in Congenital Anomaly Research

2.1 Background

Attention has focussed on drugs as potential teratogens ever since the thalidomide disaster in the early 1960s (McBride, 1961) (Leck, 1972). This disaster led to the establishment of the national congenital anomaly surveillance system in 1964 (now in the Office of National Statistics) (Office for National Statistics, 1995) in order to provide an early warning system; thus alerting the health services to a potential major problem. In addition, over the past twenty years or so a number of regional congenital anomaly registers have been established across the globe to monitor trends in the prevalence of congenital anomalies, to investigate suspected clusters and to develop research into the aetiology of congenital anomalies.

A variety of agents have been implicated in the aetiology of congenital anomalies. These are wide ranging and include physical agents eg. Xrays (Warkany, 1971), (Murphy, 1929), (Goldstein and Murphy, 1929) and environmental radiation as seen following Hiroshima and Chernobyl (Wood et al. 1967), (Satow and West, 1955); biological agents eg. the rubella virus (Leck, 1972), (Warkany, 1971); chemical agents eg. methyl mercury (Harada, 1968); and a host of risk behaviours and environmental pollutants including smoking (Kistin et al. 1996), recreational drugs (Lutiger et al. 1991), (Bingol et al. 1987), (Martinez-Frias, 1999), (Madu et al. 1999), and pollutants from land fill sites (Dolk et al. 1998) (Croen et al. 1997). Nevertheless, despite these efforts, the cause of the majority of congenital anomalies remains unknown.

The evidence for an association between thalidomide and its characteristic syndrome was overwhelming and provides an ideal example of the association between a congenital anomaly and a teratogenic drug for the following reasons (Heinonen et al. 1977):

1. The risk associated with taking thalidomide at the susceptible period of embryogenesis was large.
2. Thalidomide was used as a sedative or hypnotic drug and it was felt to be unlikely that the symptoms requiring the drug could be risk factors for the resulting malformations.
3. Phocomelia, the most prominent feature of the condition, is easily and precisely diagnosed.
4. The epidemic of the condition started with the introduction of thalidomide and terminated with its withdrawal. Prior to and following the abandonment of the use of thalidomide phocomelia was extremely rare.

As yet, other drug teratogens have been found to be less potent than thalidomide making the determination of their associated risk complex. If an agent definitely causes a condition then it is more easily identified than if the agent only sometimes causes a condition. This issue is, as in thalidomide, often complicated by the condition only resulting if exposure to the agent occurs at a particular stage of embryological development. In addition, other congenital anomalies or syndromes are more common than the main characteristic of thalidomide, phocomelia, and therefore an increase could go undetected.

2.2 Study Design

When designing a study to investigate the potential teratogenic effect of recreational drugs in early pregnancy careful consideration needs to be given to the study design. Whilst the gold standard study design to confirm causality is considered to be the randomised controlled trial, this is clearly neither appropriate nor ethical in circumstances where the exposure under investigation is not therapeutic. In studies where the investigator is neither able to control the conditions of, nor intervene in the exposure, an observational study is most robust. There are two main types of observational study available to the perinatal epidemiologist: (i) cohort and (ii) case-control.

2.2.1 Cohort Studies

The classical cohort design follows two (or more) groups of subjects who are free of disease but who differ with respect to their exposure to a suspected cause of the disease: the exposed and unexposed study cohorts. In a study where the exposure of interest is a consumptive behaviour (eg. smoking, alcohol consumption or solvent abuse) the accurate exposure status of subjects may not be easy to ascribe. It would therefore be difficult to define the cohorts for the study. In addition, rare outcomes are difficult and prohibitively expensive to investigate in a cohort study. This is because a very large cohort of births would need to be followed up in order to collect information about a very small number with the outcome of interest, as the majority of those about whom information is collected are uninformative. These major problems prohibit the use of a cohort design for studies into the cause of congenital anomalies which are rare conditions.

2.2.2 Case control studies

In studies where the outcome is rare (as in the case of congenital anomalies) the study design of choice tends to be the case control study where a group of subjects with a specified disease or outcome (cases) are compared to a group without the specified disease or outcome (controls). Exposures or risk factors thought to be associated with the aetiology of the disease or outcome are retrospectively compared between the two groups. As such the study starts with the case (diseased) and control groups and attempts to retrospectively measure one or multiple exposures. The source population from which the cases arise provides the sampling frame for the control subjects. This control group is used to determine the relative size of the exposed and unexposed denominators in the source population (Rothman and Greenland, 1998) thus allowing the estimate of relative effect measures. Although, in many case control studies actual rates cannot be calculated because only a sample of the total population of interest is being studied, in perinatal epidemiology, in particular, studies of congenital anomalies, this tends not to be a problem as total birth population data are often available. Case control studies designed to collect exposure information concerning consumption behaviours or medication use during pregnancy, after the

delivery of a fetus or infant with a birth defect, are defined retrospectively. Inherent within this type of data collection are the potential problems of reporting or recall bias. This will be discussed later.

2.2.2.1 Selection of cases

A clear definition of cases is required for this type of study, with detailed inclusion and exclusion criteria. In studies of congenital anomalies in order to include all relevant cases, cases should ideally be included from the point of diagnosis, which is often early in the second trimester of pregnancy. This ensures the inclusion of all relevant cases and prevents the exclusion of terminations of pregnancy and non-registerable late fetal losses that would reduce the power of the study but might also introduce bias. In addition the population source for the cases needs to be defined in order to be able to determine the control selection. Ideally the source would be all defined cases over a specified period of time in a geographically defined population. This would prevent selection bias arising from, for example, exposures associated with the selection of controls from perinatal tertiary referral centres where high-risk populations tend to be booked for delivery.

Ascertainment of congenital anomalies can be problematical and strategies for the identification and confirmation of all specified anomalies are required. This is facilitated in approximately half of the UK by the presence of regional congenital anomaly registers that have established multidisciplinary networks of clinical staff who regularly notify cases to the registers and confirm diagnoses.

2.2.2.2 Selection of controls

The appropriate selection of the control group is of utmost importance if the results of the study are to be valid. Basic rules for the selection of controls have been described by Rothman and Greenland (1998).

Figure 2.1: Basic rules for the selection of controls in case-control studies, adapted from Rothman & Greenland 1998.

No.	Rule
1.	Controls should be selected from the source population from which the cases arise
2.	Controls should be selected independently of their exposure status and thus be representative of the source population. This rule is modified in matched case control studies to being representative of the strata population of the matching factors.
3.	In unmatched case control studies the probability of selecting any potential control should be proportional to the amount of time that he/she contributes to the denominator of the rates that would have been calculated had a cohort study of the source population been undertaken. Controls should be sampled at a steady rate throughout the study period using the subject's exposure status at the time of sampling. Any exposure after the time of selection should be ignored.
4.	The time during which a subject is eligible to be a control should be the time in which the individual is also eligible to be a case, if the disease should occur.

However, the feasibility of control selection also has to be taken into consideration and a number of types of control have been used in studies of congenital anomalies. These control sampling methods include population, neighbourhood, random digit dialling, hospital or clinic based, other diseased or friends (Wacholder et al. 1992). The source population for studies of congenital anomalies is the total births for the defined population. A random sample selected from the total births of the population would provide the optimal control sample (Draper et al. 1999), but would only be feasible if a method for their selection were available. This process is further complicated in matched case control studies. To facilitate the selection of controls many perinatal epidemiological studies (Clarke and Clayton, 1981) have used the next delivery within the hospital following the delivery of the case as their control. As high risk pregnancies tend to be referred to tertiary centres for their care and delivery this method is likely to lead to bias, in that high risk pregnancies will be over represented in the population available for selection as controls and thus the estimated odds ratio will tend towards the null (Klebanoff and Rhoads, 1986). This bias can be reduced by adjustment within the analysis (Clarke and Clayton, 1981) or by selecting the controls from the original place of booking of the case, prior to the detection of any anomaly or problem. The latter scenario will also allow for matching to occur.

A major issue to be considered when establishing a case control study in the area of congenital anomalies is whether or not to use controls with anomalies. There

has been much controversy about this subject in the literature, the substance of which is primarily the systematic reporting differences (ie. recall bias) of exposure between cases and 'normal' controls (Paganni-Hill and Ross, 1982), (Klemetti and Saxen, 1967), (Tilley, 1985), (MacKenzie and Lippman, 1989), (Werler et al. 1989). However, there is rarely a 'gold standard' for comparison. Nevertheless, to reduce this potential differential reporting bias, malformed or 'restricted' controls have been used as an appropriate comparison group and a number of research groups recommend their use (Werler et al. 1989), (Lieff et al. 1999b). It has been postulated that mothers with malformed infants are motivated (by either guilt or concern) to remember more adverse exposures than the mothers of 'normal' infants. Werler and colleagues found that recall bias was present for some exposure factors in a study of the mothers of infants with major malformation and non-malformed infants who were interviewed during their post-partum hospital stay (Werler et al. 1989). Comparison of these data with data collected in maternal obstetric notes during pregnancy led the authors to recommend the use of controls with malformations in studies of specific congenital anomalies. Others have suggested that the use of controls without malformations is acceptable as concerns about recall bias are overrated in birth defects studies (Khoury et al. 1994).

Women with poor pregnancy outcomes have also been found to report occupational exposure more accurately than 'normal' controls (women with successful pregnancy outcome) who over-reported exposures resulting in a bias towards the null (Werler et al. 1989). Conversely, in a study where women were questioned early in pregnancy and post delivery about exposures that might influence pregnancy outcome no differential reporting bias was found to result from pregnancy outcome (MacKenzie and Lippman, 1989).

Whilst it may be considered that the use of 'restricted' controls or controls with malformations or other poor pregnancy outcomes could help eliminate differential exposure misclassification in some circumstances, it may, in fact, result in selection bias. This occurs when the malformations are affected by the exposure (Drews et al. 1993) and thus an underestimation of the effect of the exposure may occur (Pearce and Checkoway, 1988), (Miettinen, 1985). Indeed, even an

association between the exposure of interest and only a small proportion of the controls can lead to appreciable bias (Bracken, 1984).

The Slone Epidemiology Unit Birth Defects Study Group (Werler and Mitchell, 1993), (Mitchell et al. 1995) has investigated this further and looked into the differential bias that can occur in studies where cases have knowledge of the aetiological hypothesis. They have suggested that this factor should be considered in the analysis. The validity of this has, nevertheless, been questioned and it has been suggested that this should only be done when there are good reasons to believe that such knowledge would distort the recall of exposure history (Weiss, 1994). In an effort to actually quantify the effect of the use of controls with malformations a study was carried out comparing four methods of control selection taking into account the malformation type and exposure of interest (Lieff et al. 1999b). The authors concluded that, "when used selectively, infants with malformations other than the anomaly of interest can be a suitable source of controls".

This debate remains unresolved and it is left to the perinatal epidemiologist to determine the best method of control selection for the particular hypothesis in question. All possibilities for potential recall and selection bias should be taken into account in this decision.

2.3 Bias in case control studies

There are three main areas of bias that can arise in case control studies. These are referred to in a variety of ways but can be summarised into three categories as follows (Rothman, 2002):

a. Selection bias
b. Information bias
c. Confounding

2.3.1 Selection Bias

Selection bias is referred to in a number of ways in the literature including self-selection, diagnostic or response. Essentially, selection bias can arise when any factor that may influence study participation and may lead to specific exposed or unexposed groups of subjects being omitted or opting out of participation in a study or, conversely, being included in excess. This type of systematic error generally results when participants have a different association between exposure and disease to those who do not participate (Rothman, 2002). Inappropriate selection of either cases, controls or both in a case control study can lead to selection bias: eg. in a study investigating a possible association between a specific congenital anomaly and maternal smoking during pregnancy, if smokers were more likely to opt for termination of pregnancy and women who had a termination of pregnancy were more difficult to access and recruit, then the odds ratio for smoking would be underestimated.

One aspect of selection bias, self-selection, can lead to unrepresentative samples being used for studies. Self-selection may lead to highly motivated and knowledgeable subjects, who consider themselves to be at risk of specific diseases, being over-represented in studies. This may lead to an underestimate of the true population risk of disease as this group are more likely to have a non representative healthy lifestyle (Rothman, 2002). In geographically defined case-control studies of congenital anomalies, every effort is made to identify and approach all cases within the specified population along with their controls. As such there is no opportunity for individuals to self-select themselves into a study, although they can self-select themselves out of the study by becoming a non-responder.

In as far as is possible, studies need to ensure total coverage of the study subjects as selection bias limits the interpretation of study findings. Background preparation for congenital anomaly studies should therefore consider any specific issues that may affect subject recruitment and develop methods of working through these problems, for example, the approach made to subjects, dealing with issues of the sensitivity of questions and the migration of specific groups of subjects. Of

particular importance is the full co-operation of all health professionals involved in the study in order to help optimise subject recruitment and keep non-response rates to a minimum. Information about the age, sex and socio-economic details of the non-responders should be collected if at all possible to examine if there are major differences between responders and non-responders (Bhopal, 2002). In addition details about the reason for their non-response may help reduce non-response in the rest of the study. Although it should be noted that the collection of information about non-responders has become very difficult since the Data Protection Act 1998 came into force in 2000.

2.3.2 Information Bias

There are a number of aspects of information bias including recall, observation and reporting bias and differential and non-differential misclassification (Hennekens and Buring, 1987). Recall bias occurs in case-control studies when subjects are interviewed after the development of disease about their exposures over a period of time. The accuracy of recall may be directly related to the amount of time that has lapsed between the exposure and the study interview. It is crucial, therefore, that the timing of interviews for cases and controls should be the same or as near as possible to minimise differential recall in the two groups. In addition there will be a natural variation in the accuracy of memory between subjects leading to a non-differential misclassification of the data. Case control studies in perinatal epidemiology, studying birth defects or poor perinatal outcome often use interviews with mothers about their exposures and risk behaviours during their recent pregnancy. Mothers having poor perinatal outcomes have been found to report adverse exposures more accurately than those who have successful pregnancy outcomes, leading to a differential misclassification of adverse exposure data between cases and controls. (Werler et al. 1989), (Ahlborg, 1990a). This may be motivated by a maternal need to establish for themselves the cause of the poor outcome whereas the mothers of non-affected infants have no such motivation. In order to overcome this problem some studies have suggested using controls with other congenital anomalies, (Werler et al. 1989), (Ahlborg, 1990a), (Lieff et al. 1999b) see section 2.2.2.2. However, when dealing with sensitive issues such as recreational drug use, where issues of legality play a role,

then the accuracy of recall may be affected by both poor memory and deliberate omission (Fendrich and Vaughn, 1994), (Harrison, 1995).

Work has been carried out to estimate the validity and reliability of maternally reported pregnancy and delivery information, showing that this may vary with the nature of the factor of interest, (for example, the importance of an event, patients knowledge about an exposure or the way the factor is defined), but that it is little affected by time from birth or case-control status (Olson et al. 1997). Olson et al compared maternal report with information in the medical records and looked at the reliability of specific variables. Results indicated that variables such as birth weight had high levels of agreement. Similar results have been found in other studies that have shown the accuracy of the recall of birth weight even many years after an event (Axelsson and Rylander, 1984), (Lumey et al. 1994). The recall of other variables, such as whether a woman had a caesarean section or amniocentesis was also very accurate as these are considered to be important events by the women and they are easy to define (Olson et al. 1997). However, X-rays were less reliably recalled as they are seen to be of less importance. Variables based on patient knowledge or doctor-patient communication were not well remembered and the recall of symptoms was affected by increasing length of time following the event.

Similar results were found in a ten-year follow-up study of the effects of diethylstilbestrol exposure during fetal development (Tilley, 1985). A comparison of the medical records and pregnancy recall history showed a good agreement for personal history but poor recall for medical interventions such as drugs and X-rays. Almost one third of the women exposed to diethylstilbestrol were unable to recall whether they took the drug and 8% recalled that they definitely did not take it when it was recorded in their medical records. Substantial underreporting of transient illness and 'over the counter' drug use during pregnancy has also been found when comparing medical records with interview data collected following delivery (Bryant et al. 1989). This could be because women judge that this information is insignificant or that they just couldn't remember. It could also be affected by the use or lack of use of prompts during the interview process. Validation projects using the maternal medical record as the gold standard do,

however, assume that the medical record is accurate and complete. This is of course a fallacy, as all medications may not be recorded in the medical records (Harlow and Linet, 1989) and even those prescribed and recorded may not have been taken. In studies of the aetiology of birth defects, where the timing of a variety of exposures during pregnancy may be of significance, then standardised approaches towards the verification of the accuracy of recall are imperative.

Many studies have looked at the reliability and validity of data concerning risk behaviours both in the general population and in women during pregnancy (Embree and Whitehead, 1993), (Maisto et al. 1982), (da Costa Pereira et al. 1993), (Verkerk et al. 1994), (Fox et al. 1989). Although most general population studies have concluded that self-reported data on alcohol consumption are reliable and valid (O'Malley et al. 1983), (Williams et al. 1985), (Sobell and Sobell, 1978) errors do occur. It has been suggested that errors in response to questions about alcohol consumption fall into three categories: random error, wilful misrepresentation and errors in recall (Single et al. 1975). Work from Canada concluded that there are two main characteristics of questions that affect validity and reliability: the ability of a question to aid recall and its ability to mitigate the effects of respondents providing socially desirable responses (Embree and Whitehead, 1993). Social desirability and anonymity in studies can lead to the underreporting of alcohol consumption as subjects tend to give ideal or expected answers and standard confidentiality and anonymity offers seem to have no effect (Bradburn and Sudman, 1979). Methods to disguise questions (Plant and Miller, 1977), (Wilkins, 1975) and the anonymity of the use of computerised questionnaires (Skinner and Allen, 1983) have also been found to have no effect.

EUROMAC (da Costa Pereira et al. 1993), a longitudinal study following women before during and after pregnancy to investigate the effect of maternal alcohol consumption on birth weight, concluded that differences in reporting could be accounted for by women's ignorance of their actual intake, deliberate misreporting or by differences in the way the data were collected (self completed questionnaire, telephone interview or face-to-face interview). In a study of 700 pregnant smokers enrolled in a randomised controlled trial of a smoking cessation intervention the reliability of self reports of smoking and alcohol consumption was found to be high

(Fox et al. 1989). A test re-test method was used in early pregnancy (before 18 weeks gestation) and in the 8th month of pregnancy. Although only half of the subjects gave an identical report at the test and re-test, in those who changed their responses, the change was very small.

A Dutch study (Verkerk et al. 1994) of the differential misclassification of alcohol and cigarette consumption by pregnancy outcome (normal births, stillbirth, small for gestational age, congenital malformations, preterm birth and low birth weight) found that recall bias was unlikely to have a large influence on effect estimates in studies using retrospective information on alcohol and cigarette use. Data were collected both prospectively, at around the 18th week of gestation and retrospectively following delivery. The only statistically significant finding from this study was found for the association between cigarette smoking and small for gestational age (SGA) infants, where their mothers reported a higher number of cigarettes smoked retrospectively than they had prospectively, a finding more common in the mothers of SGA infants than in the control mothers. However, the odds ratios for SGA infants in smoking mothers based on the prospective and retrospective data were virtually the same and the authors concluded that the impact of information (recall) bias on effects estimates is limited.

Pregnant women in Toronto who are concerned about drug and chemical exposures during early pregnancy are invited to attend the Motherisk Program where they receive counselling about the risks associated with the exposures. In 1985 a study of 145 consecutive pregnant women attending for counselling were studied following delivery to measure the accuracy of their recall of drug and chemical exposure (Feldman et al. 1989). No difference in recall was found between women having normal compared to adverse pregnancy outcome. However, numbers were small (112 and 33 respectively). Accurate recall of the toxic substance or teratogen which led to their attendance at the program was high (81%), as was smoking recall (79%). Recall of alcohol exposure was poorer (59%). In addition there was an inverse correlation between the number of exposures and the mother's recall: 85% of mothers recalled correctly one exposure compared to 40% who recalled four exposures. Older women (30 years or more) had significantly worse recall than their younger counterparts: 52%

compared to 70%. Overall women recalled less alcohol consumption than had been initially reported.

The main difference between the risk behaviours already discussed and recreational drugs is that the use of recreational drugs is illegal. This adds an extra layer of complexity to the collection of such data especially in the willingness of the subject to admit using these drugs. Early non pregnancy related studies, prior to the mid 1980s, suggested that drug use was fairly well reported when comparing urinalysis with interviewed data (Bale et al. 1981), (Cox and Longwell, 1974), (Page et al. 1977), in up to 90% in some studies. However, since biochemical methods have been improved and used routinely by criminal justice systems to validate self-reported recreational drug use less concordance has been found, with only between one-third to one-half of users in major US cities admitting recent drug use (National Institute of Justice, 1997). Factors found to affect the reporting of recreational drug use are how recently the drug was consumed, the social desirability of the specific drug (ie. what class of drug it is) and the method of data collection (Harrison, 1995), (Colon et al. 2001). Urinalysis is limited to drug use within the past few days and therefore has limited value in studies where retrospective data are collected about drug use in early pregnancy or over any longer period of time. Hair analysis overcomes this limitation.

Much of the work carried out looking at the correlation between self-reported drug use and biochemical detection uses high-risk populations for drug use. Small studies of known drug dependents show high correlations between self reported drug use and biochemical validation methods (Elman et al. 2000). However as the subjects self-selected themselves for these studies, the study results are unlikely to be representative of drug dependents as a whole as this group are more likely to be compliant. In a larger study of 322 subjects from a high risk community sample in Chicago hair specimens were collected to validate survey responses about recent and lifetime drug use (Fendrich and Johnson, 1999). The estimated prevalence of cocaine use from the hair analysis (allowing a cut-off for passive exposure) was five times higher than past month reporting and four times higher than past year reporting, suggesting a high level of under-reporting (Colon et al. 2001).

Studies from the USA concerning the accuracy of reporting recreational drug use by pregnant women have indicated that a large proportion of these women deny the use of such drugs; 89.5% with cocaine metabolites present in serum denied using cocaine and 69.2% with marijuana metabolites in their serum denied using marijuana (Shiono et al. 1995). Validation studies using hair analysis have also found large proportions of test positive pregnant women denying the use of cocaine. In a New York hospital study of 397 pregnant women, 32% reported ever use of cocaine whereas 59% of hair sample tests were positive (Kline et al. 1997) with samples from self-reported drug free individuals having values exceeding three standard deviations above the mean (Baumgartner et al. 1989). In a study comparing self-reported and biochemical measures for tobacco, marijuana and cocaine exposures among women in pregnancy, women with positive biochemical tests were as likely to deny use of tobacco as they were to deny marijuana or cocaine (Markovic et al. 2000). Urinary analysis was equally likely to be positive in women reporting never use as those reporting past use for tobacco, marijuana and cocaine. When comparing hair analysis results with urine testing for cocaine, four times as many exposures were identified among women who reported never using cocaine. These studies suggest that in order to obtain an accurate prevalence of the use of recreational drugs in pregnant women, validation of self-report information should be carried out using a biochemical method (Grant et al. 1994).

2.4 Confounding

Elwood (1998) defines confounding as "a distortion of an exposure-outcome association brought about by the association of another factor with both outcome and exposure". In observational studies there could be an important influence/factor that may have an effect on the outcome that differs systematically between the comparison groups (Clayton and Hills, 1993). In a controlled experiment confounding can be prevented by randomising the subjects into study groups. However this is not possible in the type of epidemiological studies used in congenital anomaly research. Three criteria must be fulfilled if a variable is to be considered as a confounder (Rothman and Greenland, 1998). A confounding

factor must be a risk factor for the disease or condition; it must be associated with the exposure under study in the source population; and, it must not be affected by the exposure or the disease i.e. a confounding factor must not be an intermediate step in the causal pathway between the exposure and the disease (Grayson, 1987), (Weinberg, 1993). In epidemiological studies adjustment is often made for factors that are not on the causal pathway but are caused in part by the exposure. However, bias can result when adjustment is made for any factor that is caused in part by the exposure under study and is also correlated with the outcome under study (Weinberg, 1993).

There are methods available to the epidemiologist to try and minimize confounding within study design. These are the use of randomisation (which can only be used in an experimental design), restriction or matching (Rothman, 2002). Restriction involves the selection of subjects for a study with the same (or similar) value for a specific variable that is thought to be a confounder, for example, to restrict the study to men only. This method is rarely used in case control studies because the restriction of the admissibility criteria can reduce the, already limited, number of available subjects for the study, to below the required figure. Matching is more complicated and is discussed in the next section. However, before any of these methods can be used work should be carried out to identify and collect information about any potential confounding factors in a study. One basis for any such identification is an understanding of the potential causal pathways linking the events being studied (Hernan et al. 2002).

In studies investigating the association between maternal medication use during pregnancy and birth defects, one important source of confounding is the reason for the administration of the drug. The underlying maternal conditions should therefore be taken into account because if the condition is, in fact, the cause of the birth defect, an apparent association with the drug will be present. Confounding arising from this cause is unlikely to be a factor for maternal recreational drug use except in the more unlikely scenario of marijuana being used for pain relief in specific conditions such as multiple sclerosis.

2.4.1 Matching

One way of dealing with the effects of confounding in the design of epidemiological studies is to match the controls to the cases on those variables for which there is strong evidence that they confound the relationship between the exposure of interest and the outcome (Rothman and Greenland, 1998). In case control studies, because 'case-ness' depends upon disease outcome then the effect of matching only affects the controls. If the matching variable is associated with the exposure of interest (one of the determinants of a confounder) then the controls will inevitably be similar to the cases and the overall result will be a reduction in the odds ratio for the exposure. Analysis of such studies either using stratification of the matching variable or treating the matching variable as a confounder can eliminate this bias. The introduction of these effects into case control studies means that a matched study design should be considered very carefully and used only where there is no alternative. The major aim of matching in case control studies is, therefore, to facilitate comparisons that are relatively free from bias that might arise from basic dissimilarities of the case and control populations (Lilienfeld and Lilienfeld, 1980). Thus, in circumstances where there is very little overlap between cases and controls for a confounding factor, matching is required. An example of this is in studies of cerebral palsy. Cerebral palsy is strongly associated with pre-term birth. Gestational age may well be correlated with other major factors such as ante partum haemorrhage and congenital anomalies and will therefore have an effect upon the analysis. If controls are not, therefore, matched to cases on gestational age then the distributions of gestational age between cases and controls will be very different with very few controls at the lower gestational ages and very few cases at around term. The effect of this lack of overlap between the two groups would be to give a very imprecise estimate of the effect of gestational age on cerebral palsy. Therefore in order to populate the lower gestational age strata to allow for analysis the control sample would need to be matched to the cases' gestational age. In this way matching will deal with the potential problem of no overlap on a particular characteristic by forcing a directly matched overlap if the matching is complete.

There are a number of disadvantages of matching in case control studies. One major disadvantage is that once a factor has been used for matching purposes it can no longer be used in the analysis and therefore the effect of that factor cannot be estimated (Rothman and Greenland, 1998), (Elwood, 1998). If incomplete control selection occurs then any cases without matched controls must be excluded from the analysis. In addition, the matching used in a study can lead to overmatching, the inadvertent matching on other factors that may be of interest, thus leading to selection bias (Bhopal, 2002).

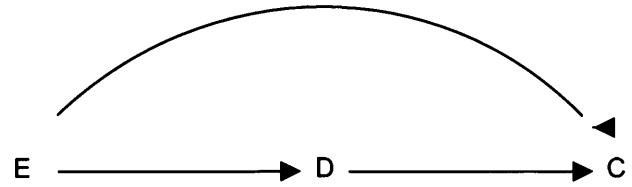
2.4.2 Analysis issues and confounding

The analysis of matched case control studies is limited to an analysis of discordant pairs or case control sets (Breslow et al. 1978) thus limiting the information available for analysis. If a matched control subject has not been available in a 1:1 matched case control study then the case pair will not be able to be used in the analysis, however, if one or more of a multiple control matched set was not available then so long as at least one of the controls is available the analysis can be carried out using varied numbers of matched controls. Residual confounding may still be present if the matching variables for the study were imprecisely matched and these issues should be clarified prior to the study analysis so that any issues of bias may, if possible, be addressed.

The use of a stepwise selection (i.e. one by one addition or removal) of potential confounders in analysis can lead to variables being selected that are not confounders and the assumption that all confounders have been selected. Other statistical associations can be investigated by comparing unadjusted and adjusted odds ratios. However, none of these techniques necessarily uses basic biological data about the associations between exposure, outcome and any potential confounders and this is therefore the main limitation of many epidemiological studies. Causal diagrams can facilitate the process of confounder identification leading to the appropriate stratification of adjustment of the variable and thus eliminating the spurious component of the association between exposure and disease (Greenland et al. 1999), (Robins, 2001), (Hernan et al. 2002). Two examples taken from Hernan et al (2002) are given in Figures 2.2 and 2.3. Figure

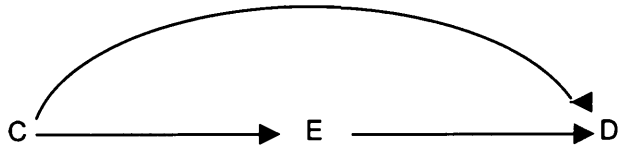
2.2 shows a situation where there is no confounding. The exposure (E) and the disease (D) do not share common causes and their association is wholly due to the causal effect of the exposure on the disease. In Figure 2.3, however, the exposure and the outcome (disease) share a common cause (C), which is acting as a confounder.

Figure 2.2 Causal diagram example where there is no confounding.



Low folate intake (E) may increase the risk of preterm delivery and infant low birth weight (C) and many birth defects (D) result in preterm deliveries and low birth weight infants.

Figure 2.3 Causal diagram example where there is confounding.



Multivitamin use (E) may reduce the risk of certain birth defects (D), and maternal age (C) may affect multivitamin use.

2.5 Standardisation of data collection

All aspects of the collection of data in epidemiological studies are very important. Once the study design has been chosen the method of data collection and any data collection instruments need to be developed. Data may be collected in a number of ways including the use of routinely collected data, medical notes abstraction, self-completion questionnaires or interviews over the telephone or face-to-face. The choice of the mode of data collection depends upon a number of factors including the data that are actually required, cost implications, amount of time available and any data validation requirements. In addition, the mode of data collection will have a direct effect upon the response rate for the study where subjects are being approached to participate, with postal questionnaires tending to have lower response rates, from around 50% to 80% (Cartwright, 1988), (McHorney et al. 1994), (Mickey et al. 1994), (Fox et al. 1992), (Brambilla and McKinlay, 1987), (O'Toole et al. 1986), (Siemiatycki, 1979) and face-to-face interviews optimising response rates to in excess of 95% (Donovan et al. 1997), (Cartwright, 1988), (McHorney et al. 1994), (Mickey et al. 1994), (Fox et al. 1992), (Brambilla and McKinlay, 1987), (O'Toole et al. 1986), (Siemiatycki, 1979), (Draper et al. 1999). As reported in section 2.4.2 on information bias, data may be collected from different sources for validation purposes, for example, to use data from medical notes to validate data collected at face-to-face interviews. In studies requiring the collection of biological specimens for validation purposes, face-to-face contact with the subject is required, providing the ideal opportunity to carry out a face-to-face interview and optimise the study response rate.

Many epidemiological studies use a standardised questionnaire for the data collection instrument. The structure and content of the questionnaire require careful consideration and validation. Three types of question are available for use: those that collect factual information, those that ask for the opinion of the subject or those that test the knowledge of the subject. These questions can be asked as open-ended questions or as structured questions that provide a specified range of answers. Studies investigating the aetiology of diseases or conditions such as congenital anomalies, tend to concentrate on factual questions of a predominantly

structured format (Werler et al. 1999), (Erickson, 1991), (Hernandez-Diaz et al. 2000). Where possible the use of validated questions and scales are advised. However, if new questions are being developed they need to be piloted to ensure their face validity, understandability and appropriateness (Abramson and Abramson, 1999) with both study subjects and by data collectors who are abstracting data from routine sources or medical records. The format and design of a questionnaire needs to be kept as interesting and logical as possible, especially in circumstances where the subject is completing the questions themselves. Overly long questionnaires are very time consuming and boring to complete, thus care should be taken to ensure that questionnaires are succinct and as short as possible, whilst collecting the data required.

Rigorous training of data collectors and interviewers is required to ensure standardised questioning techniques and data recording and to minimise inter-observer and intra-observer variability (Abramson and Abramson, 1999), (Donaldson and Donaldson, 1993). A data collection manual should be prepared with clear instructions for questionnaire completion, definitions of all variables and details of how to record answers. Interviewers need to be familiar with the questionnaire, providing information for the respondent about what is required, thus enabling them to answer the questions. The logical flow and sensitivity of the questionnaire is important as the order of questions can affect responses (Abramson and Abramson, 1999). In addition, it is useful to have an agreed introduction for different subject areas as they arise within the questionnaire. Subjects need to be motivated firstly to agree to participate in an epidemiological study and secondly to maintain their concentration throughout the length of the questionnaire. The timing of an interview may have an affect upon motivation as subjects may be distracted by others being present or the demands of the home, for example, around mealtimes or school finishing times. Interviewers themselves may have a direct effect upon the motivation of their subjects by their body language, empathy, tone of voice and manner. Judgmental mannerisms from the interviewer can lead to the underreporting of risk behaviours and the memory of illnesses being exaggerated (da Costa Pereira et al. 1993), (Harrison, 1995). Interviewers need therefore to remain and appear neutral. Probing is a useful tool to help with the collection of data in sensitive areas or requiring long term recall.

Structured interviews with standardised flashcards and checklists can help in these situations as well as pre-agreed prompts (Hayes et al. 1996), (Louik et al. 1987). In studies of medication use during pregnancy the accuracy of maternal recall can be improved by asking the women to search for their medications used during pregnancy (Hayes et al. 1996) and by using a calendar marked with the date of the mothers' last menstrual period (Hernandez-Diaz et al. 2000). Women may judge particular drugs or illnesses to be insignificant and therefore, if they are not prompted for this information by the interviewer, this information may be missed (Bryant et al. 1989). In this way study designers can bias the data collection for a study by only concentrating on those drugs or illnesses they believe to be of significance.

The techniques required for the collection of biological samples, for example hair, for validation purposes should be part of any training programme for data collectors. Issues concerning the risks associated with this sample collection and sensitivity surrounding the samples should be clearly addressed. When collecting these samples data collectors/interviewers need to be aware of the affect they have upon the subject and learn to put the subject at ease.

2.6 Modelling and variable selection in epidemiological analysis

In case control studies the main outcome measure or measure of association is presented as an odds ratio with a 95% confidence interval. The odds ratio measures the strength of the association between an exposure (eg. smoking) and a disease (eg. birth defects), providing information that can help determine whether any observed association is causal (Hennekens and Buring, 1987). A univariate odds ratio provides information about an association without any adjustment for confounding factors. One way to adjust for these factors is to develop a model. However, prior to the development of a model to estimate effects, it is necessary to evaluate the assumptions made by the model against both the data and any prior information including the biological plausibility (Greenland, 1989), (Clayton and Hills, 1993). This will reduce any inappropriate adjustments for factors on the causal pathway. Graphical techniques and simple

calculations can help in the understanding and plausibility of the data analysis (Bland and Altman, 1986).

To allow for potential confounding and effect modification stratification, standardisation and multivariate techniques can be used to estimate the odds ratio associated with the risk factor/exposure in question (Rothman and Greenland, 1998). Tests of significance may be used to evaluate the role of chance, however, care must be taken not to over-analyse data as there are limitations to interpreting statistical significance. Work carried out looking at the problems of multivariate modelling and variable selection in epidemiological analyses (Greenland, 1989) has concluded that: a) model and variable forms should be selected based on regression diagnostic procedures as well as 'goodness of fit' tests; b) variable selection algorithms such as stepwise regression can lead to invalid estimates and tests of effect; and c) variable selection is better approached by direct estimation of the degree of confounding produced by each variable rather than by significance-testing algorithms. Tests for trends and interactions between variables should form part of the model development process (Clayton and Hills, 1993). Finally, the robustness of any final model and the associated odds ratios should be checked for issues of leverage and influence by investigating the effect of any outliers.

2.7 Attributable risk

By combining information about the distribution of an exposure with the estimated odds ratio the size of the proportion of subjects with a specific disease in the population explained by the exposure can be estimated (Breslow and Day, 1992), (Donaldson and Donaldson, 1993). This population attributable risk is a useful tool in the assessment of the public health impact of the exposure of interest indicating the number of cases with a specific disease that could be prevented by this exposure (Hennekens and Buring, 1987). However, as the name suggests, the attributable risk makes the assumption that the link between the exposure of interest and the specified disease is causal (Breslow and Day, 1992) and the interpretation of attributable risks assume that biases are absent (Rothman and

Greenland, 1998). Caution should therefore be exercised in their interpretation. The precision of the estimate of the attributable risk is determined by the level of uncertainty of the odds ratio and the prevalence of exposure in the population. Greenland (1989) has suggested a method of estimating the variance of the attributable risk that allows for sparse data. Bayesian approaches to measuring the uncertainty associated with attributable risk have also been suggested (Abrams et al. 1998).

2.8 Summary

The study design of choice for congenital anomaly research is the case-control study. Cases should be clearly defined and every effort made to ensure their complete ascertainment and minimise selection bias. The appropriate selection of a control group is of utmost importance and consideration should be given to the question of whether or not to use controls with anomalies. Strategies should be developed to minimise information bias including the use of biochemical validation techniques for self-report information concerning exposures such as recreational drug use. If a factor is known to confound the relationship between the exposure of interest and the congenital anomaly being studied then a matched case control study may be undertaken. Following the calculation of univariate odds ratios for the risk factor of interest and any possible confounding factors a multivariate model can be developed to include those factors considered to be biologically plausible. Results from the final multivariate analysis model can then be used to calculate the proportion of cases of the congenital anomaly being studied that may be attributable to the exposure in question.

Introduction: Part Three – Recreational Drug Use

3.1 Background

Substances that modify the state of consciousness have been used throughout history, their social acceptability often changing over time or by location. One such example is alcohol, which is totally accepted in the Western world but banned in some Moslem countries. Another is laudanum (a solution of opium in alcohol), which, in the early nineteenth century, was readily available for everyday use, whereas today it is classified as an illicit or illegal drug in most parts of the world. For the purposes of this study the term 'recreational drug' will be used to describe those drugs that are currently illegal (outside medically prescribed circumstances) in the UK (see Figure 3.1).

Figure 3.1 Current status (class) of recreational drugs in the UK

Recreational Drug	Designated Class in UK (2004)
Heroin	Class A
Ecstasy (3,4 methylenedioxyamphetamine)	Class A
Cocaine / Crack	Class A
LSD (lysergic acid diethylamide)	Class A
Amphetamines	Class B
Cannabis	Class C

There has been a steady increase in the use of recreational drugs since World War II and recently the consumption of recreational drugs has reached epidemic proportions. Indeed, it is estimated that some 45 million European Union citizens have used cannabis at some time, with proportionately higher use among young people (European Monitoring Centre for Drugs and Drug Addiction, 2001). Similarly, class A drug use (cocaine, heroin etc) is also rising in the EU with an estimated 1.5 million users.

Recreational drug use is now a major problem, worldwide, especially in the USA where there have been dramatic increases. Estimates of cocaine use in the 1980s and early 1990s suggest that 20 million Americans had tried cocaine and between six and ten million were regular users (Matti and Caspersen, 1993). Worryingly, the mean reported age for recreational drug use also reduced over this period, for example, in 1988 the mean reported age for heroin use in the USA was 27 years which had reduced to only 19 years by 1995 (NIDA, 1997). As a result there has been a significant increase in the use of these drugs by school children. Heroin use in 15 to 16 years olds in the USA increased from 0.9% to 1.8% over the period 1990 to 1996 and then showed a further increase to 2.1% in 1997 (Schwartz, 1998), (Johnston et al. 1997).

International comparisons of the prevalence of recreational drug use are fraught with problems. These include the methods of measurement, quality of data collection, validation of the data and standardisation. There are, however, similar patterns of recreational drug use emerging around the world (United Nations Office on Drugs and Crime, 2003)).

3.2 Prevalence of recreational drug use in Britain

National data concerning the use of recreational drugs in England and Wales are collected as part of the British Crime Survey, (Ramsay and Percy, 1996), (Ramsay and Spiller, 1997), (Ramsay and Partridge, 1999), (Aust et al. 2002), (Condon and Smith, 2003), (Ramsay et al. 2001), using the adults in a representative cross-section of private households. Hostels, care homes and universities are not, however, sampled. This study asks subjects about their experiences of crime and has, since 1994, contained a self-completion module asking a number of questions about recreational drug use in those aged between 16 and 59 years of age. Recent measures of the response rate for this study run at around 74% (Aust et al. 2002), (Condon and Smith, 2003).

The BCS started out as a biennial survey but since 2002/3 has become a continuous survey covering financial years. In addition, the 2002/3 BCS included a booster sample of 16 to 24 year olds for the recreational drug use questions, in an effort to provide information for the Government drug strategy for young people. A total of 4292 young people aged 16 to 24 years participated in this part of the study in 2002/3 with a response of 75%. Since 1996 the analysis of the BCS data has included a calibration weighting that adjusts for known differentials in response rates across age, gender and regional sub-groups (Bolling et al. 2002).

Table 3.1: Prevalence (%) of any illicit drug use in the last year and last month, adapted from British Crime Surveys 1994 – 2002/3.[1]

Age-group	Time period	1994	1996	1998	2000	2001/2	2002/3
16-19 years	**Last year**	34	31	31	27	28	27
	Last month	20	19	22	16	17	17
20-24 years	**Last year**	25	27	28	30	31	30
	Last month	15	18	17	29	20	19
25-29 years	**Last year**	15	17	19	20	na	na
	Last month	9	10	11	12	na	na
16-59 years	**Last year**	10	10	11	11	12	12
	Last month	na	6	6	6	8	7

na - not available in the BCS publications

Whilst the BCS shows an increase in the overall trend in recreational drug use in all age-groups, over the period 1994 to 2002/3 (Table 3.1), the use of any recreational drug in the last year decreased in the youngest age-group (16 to 19 years) from 34% to 27%. However, in those aged 20 to 24 years a steady increase in the use of any recreational drug in the past year, from 25% to 30% occurred. A similar pattern can be seen for the use of any recreational drug during the past month with a reduction from 20% to 17% in the 16 to 19 year age-group compared with an increase from 15% to 19% in those aged 20 to 24 years.

[1] (Ramsay and Percy, 1996), (Ramsay and Spiller, 1997), (Ramsay and Partridge, 1999), (Ramsay et al. 2001), (Aust et al. 2002), (Condon and Smith, 2003)

These figures are indicative of a stabilisation of the prevalence of any recreational drug use in the immediate post-millennium period following the increase in the 1990s, with the highest levels of any recreational drug use for those under 25 years of age.

The majority of those aged 16 to 24 years who reported recreational drug use, used a single drug alone; 74% in the last year and 62% in the last month. The use of two drugs was reported by 17% of this group in the last year and 12% in the last month. Reducing proportions of this group reported higher levels of polydrug use with the use of six or more drugs being reported by 4% in the last year and 2% in the last month.

Table 3.2: Prevalence (%) of any class A drug use in the last year/last month, adapted from the British Crime Survey 2000 – 2002/3.[2]

Age-group	Time period	2000	2001/2	2002/3
16-19 years	**Last year**	8	6	6
	Last month	5	3	3
20-24 years	**Last year**	10	11	10
	Last month	5	7	5
16-59 years	**Last year**	3	3	3
	Last month	1	2	2

Recent prevalence estimates for class A drug use are provided in Table 3.2. A small reduction in the use of any class A drug, over the past year and past month, occurred in the youngest age-group over the period 2000 to 2002/3 (8% to 6% and 5% to 3%, respectively) with a stabilisation of class A drug use in the 20 to 24 year age-group (10% and 5%, respectively). Those aged 20 to 24 years are most likely to have ever tried class A drugs including amphetamines, cocaine and ecstasy (4.6%, 6.4%, 6.9%, respectively).

[2] (Ramsay et al. 2001), (Aust et al. 2002), (Condon and Smith, 2003)

Data, from the BCS in 2000, show that men are more likely to report the use of recreational drugs than women (Table 3.3). However the ratio of male to female recreational drug use decreases with age from a two-fold difference in the 30 to 59 age-group for use within the last year compared to a 1.3 fold difference in both the 16 to 19 and 20 to 24 age-groups.

Table 3.3: Percentage of respondent using drugs in the last year and the last month by age-group and sex (BCS 2000).[3]

	Age-group in years					
	16-19	**20-24**	**25-29**	**16-29**	**30-59**	**16-59**
Last year						
Men	31	34	26	30	8	14
Women	24	26	14	20	4	8
All	27	30	20	25	5	11
Ratio men/women	1.3	1.3	1.9	1.5	2.0	1.8
Last month						
Men	21	23	19	21	4	9
Women	12	16	6	11	2	4
All	16	20	12	16	3	6
Ratio men/women	1.8	1.4	3.2	1.9	2.0	2.3

Other factors reported in the BCS to show variations in the levels of recreational drug use are region of residence, type of neighbourhood and ethnic group. Overall the BCS 2001/2 showed that 12% of people in England and Wales aged 16 to 59 years had taken a recreational drug in the last year. In the London Government Office region, however, this proportion was significantly higher at 16% compared to those living in the North East, Wales and the East Midlands regions where the

[3] (Ramsay et al. 2001)

proportions were significantly lower: 7.5%, 8% and 9%, respectively. A similar pattern was found when looking at class A drug use alone. The highest level of any recreational drug use during the past year was found in affluent urban areas (22%). This compares to 14% in inner city areas and 9% in affluent family areas as defined by 'A Classification of Residential Neighbourhoods' (ACORN), a classification that groups households by demographic, employment and housing characteristics based on the 1991 census data. In order to describe the patterns of use of recreational drugs in the ethnic minority population the BCS 2001/2 included a booster sample of black and minority ethnic respondents. Levels of recreational drug use in the 16 to 24 age-group were highest in the mixed ethnic group: 26% last year use compared to 12% in both the white and black groups and 5% in the Asian group. Use of class A drugs in the last year followed a similar pattern although levels of use were slightly higher in the white group than the black group (3% and 2%, respectively, with 7% in the mixed group and 1% in the Asian group).

In recent years the Office for National Statistics has carried out an annual survey, in English schools, estimating the prevalence of smoking, alcohol consumption or recreational drug use in 11 to 15 year olds (Boreham and McManus, 2002). In 2002 the proportion of these young people reporting the use of recreational drugs in the past month was 11% and 18% in the last year. These proportions were higher in boys (12% and 20%, respectively) than in girls (9% and 17%, respectively). The prevalence of reported recreational drug use in the past month increased with age from 3% in 11 year olds increasing to 22% of 15 year olds. The most widely used drug was cannabis with 13% of pupils having taken it in the past year, ranging from 1% of 11 year olds to 31% of 15 year olds. The prevalence of class A drug use in the past year showed a similar, though smaller increasing gradient with age: 1% in 11 year olds to 8% in 15 year olds.

Combined together these national data suggest that the prevalence of the use of recreational drugs increases steadily from age 11, peaks at 20 to 24 years of age and then declines fairly rapidly thereafter (Ramsay et al. 2001), (Aust et al. 2002), (Condon and Smith, 2003), (Boreham and McManus, 2002). Males are more likely to participate in recreational drug use at all ages, but more so after the 20 to 24

age-group peak in use. The highest prevalence of recreational drug use is found in those of mixed ethnic background and also affluent urban or inner city dwellers. However, all these data are from self-report alone and, as such, may underestimate the size of the problem.

3.3 Prevalence of recreational drug use in pregnancy

There is a paucity of UK data available concerning the prevalence of recreational drug use during pregnancy. The only data identified come from the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC), (Golding and ALSPAC Study Team, 1996), which were collected over the period April 1991 to December 1992 and reported on cannabis use alone. Over 12,000 pregnant women returned the self-completion questionnaire, which included questions on recreational drug use, sent out to them in mid-pregnancy, (Fergusson et al. 2002). Of these, 4.8% reported using cannabis six months before pregnancy, 2.6% during the first trimester and 2.1% in mid-pregnancy. Compared to mothers who did not report cannabis use mothers who reported using cannabis were younger (mean age 25.5 years compared to 27.8 years in non users), of lower parity, better educated and more likely to be cigarettes smokers and to drink alcohol and caffeinated drinks. Cannabis users were also more likely to report the use of other recreational drugs: 6.8% of users compared to 0.2% of non-cannabis users. However, although this study is population based it is limited by the fact that the data are based on maternal self-report information alone.

Two European studies have addressed the issue of validation of self-report data but are focused on hospital-based samples and therefore may have limited generalisability. A small study in Dublin estimated the prevalence of recreational drug use in their obstetric population by anonymously testing the urine of 504 'first visit' antenatal attendees (Bosio et al. 1997). A separate sample of 515 patients attending for their six-week postnatal visit also had their urine tested anonymously for the presence of recreational drugs. The prevalence of recreational drug use from the urinary analysis was 2.8% for the antenatal population and 5.6% for the post-natal population. Recreational drug users were more likely to be single,

unemployed and to have had a previous pregnancy. None of the test positive women had been formerly identified as a user. In Barcelona the prevalence of cocaine use during pregnancy was estimated in 1773 women who delivered in one hospital (Martinez Crespo et al. 1994). All women completed a structured questionnaire about their use of recreational drugs during pregnancy up to the time of delivery. Urine samples were routinely collected before delivery and tested for the presence of heroin and cocaine. The prevalence of heroin and cocaine use from the questionnaires was 1.3% compared to 2.5% from the urinary analysis, indicating a substantial level of denial in these women.

Many American studies have been carried out to estimate the prevalence of recreational drug use during pregnancy using toxicological analysis to validate maternal self-report data (Frank et al. 1988), (Gilchrist et al. 1996), (Lester et al. 2001), (Matera et al. 1990), (Neerhof et al. 1989). These studies have, however, focused on high-risk populations of pregnant women and tend to be based on small samples. Results from these studies indicate high levels of cocaine use during pregnancy of up to 11% for inner city pregnant women (Frank et al. 1988), (Matera et al. 1990), delivering very low birth weight infants (Lester et al. 2001) and for unmarried pregnant adolescents (Gilchrist et al. 1996). Wide variations have been found in the levels of agreement between self-reported recreational drug use and toxicological analysis with between 11% and 24% of the pregnant women denying the use of cocaine but having a positive toxicological test (Lester et al. 2001), (Frank et al. 1988). It would appear, therefore that there is no generalisable population based data available for pregnant women in the USA.

3.4 Impact of recreational drug use on general health

Over and above the state of euphoria, intoxication, 'highs' or 'lows' associated with the use of recreational drugs there are a number of longer-term health consequences. With the majority of class A drugs there is an associated physical or psychological dependence whereas with other drugs, for example, cannabis, the addictive effects have not been proven. A problem with many of the commonly

used recreational drugs is that the user is never certain of the purity of the drug and therefore the amount consumed. The most serious consequences of this uncertainty are overdose and, in the worst cases, death.

The carcinogens produced when smoking cannabis have the potential to result in approximately five times the amount of lung damage than that caused by smoking ordinary cigarettes (Wu et al. 1988). Other suggested health risks associated with cannabis use include tachycardia and other cardiac problems exacerbated by salt and water retention, suppression of the immune system and a possible reduction in the production of sex hormones, ovulation and spermatogenesis (Ghuran et al. 2001), (Jones, 2002), (Rezkalla et al. 2003), (Smith and Asch, 1984), (Fody and Walker, 1985), (Brown and Dobs, 2002). Toxic psychosis has also been found to occur following heavy or prolonged cannabis use, although there is no proof that it causes schizophrenia in a non-predisposed person (Thomas, 1993).

The use of 3,4-methylenedioxymethamphetamine ('ecstasy') is associated with numerous minor effects including tachycardia, mood disturbance, nystagmus and abnormal gait, residual mood disturbance and insomnia (Cook, 1995), (McCann et al. 1996). Serious physical reactions can occur following consumption (Henry et al. 1992) including sudden death, malignant hyperpyrexia with disseminated intra-vascular coagulation and renal damage, and also liver failure (O'Connor, 1994). Intra-cerebral haemorrhage has also been noted in a number of cases (Harries and De Silva, 1992) as well as cardiac arrythmias (Dowling et al. 1987), hepatotoxicity, acute and chronic psychiatric state and neurological conditions (Henry et al. 1992), (O'Connor, 1994), (McGuire and Fahy, 1991).

Cardiovascular complications are also associated with prolonged cocaine use. Cardiac arrhythmias, ventricular fibrillation, myocardial infarction, pulmonary oedema, hypertension and circulatory collapse have all been recorded in habitual cocaine users (Nanji and Filipenko, 1984), (Coleman et al. 1982), (Ghuran et al. 2001), (Barroso-Moguel et al. 1991). Other major health problems in cocaine users include convulsions, hyperpyrexia and psychosis (Lowenstein et al. 1987).

The standardised mortality rate associated with heroin use has been found to be up to seven times higher than the general population rate and the cause of half of the excess deaths are due to the direct effects of heroin consumption (Joe and Simpson, 1987). One acute adverse effect of heroin use is respiratory depression caused by the direct suppression of the brain stem respiratory centre (Schwartz, 1998) which can ultimately lead to death.

Amphetamines are generally inexpensive but tend to be impure. Psychosis and other psychological symptoms including depression, anxiety, paranoia, hallucinations and violent behaviour are often associated with amphetamine use (Hall et al. 1996). Structural changes to the brain have been found following the long-term use of amphetamines (National Institute on Drug Abuse, 1998). Methamphetamine dependence often leads to deficits in selected neuropsychological domains, including memory, learning and executive systems functioning (Kalechstein et al. 2000). As with other recreational drugs increased cardiac problems may occur, including arrythmias and cardiomyopathy (Ghuran et al. 2001).

3.5 Effects of recreational drug use in pregnancy

A number of complications may arise as a result of taking recreational drugs during pregnancy. These include spontaneous abortion, premature onset of labour and rupture of the membranes, abruptio placenta, preterm delivery and stillbirth (Ness et al. 1999), (Delaney et al. 1997), (Neerhof et al. 1989), (Bingol et al. 1987), (Miller et al. 1995), (Burkett et al. 1994), (Lampley et al. 1996), (Kistin et al. 1996), (Feldman et al. 1992), (MacGregor et al. 1987), (Singer et al. 1994). In addition medical and developmental problems may result in the fetus and infant including intra-uterine growth restriction, small head circumference and an increased risk of necrotising enterocolitis, intra-ventricular haemorrhage and sudden infant death ((Fox, 1994), (Castro et al. 1993), (Cherukuri et al. 1988), (Chiriboga et al. 1999), (Bateman and Chiriboga, 2000), (Feng, 1993), (Czyrko et al. 1991), (Singer et al. 1994), (Kandall et al. 1993), (Davidson-Ward et al. 1992)). Evidence of an association between recreational drug use in pregnancy

and congenital anomalies thought to result from vascular disruptions (eg. gastroschisis, small intestinal atresia, porencephaly) have been reported (Martinez-Frias, 1999); (Werler et al. 2003); (Torfs et al. 1994) as well other links with other anomalies (Bingol et al. 1987), (Martinez-Frias, 1999), (Fox, 1994), (Chasnoff et al. 1988). In the longer term childhood behaviour problems (Chatterji and Markowitz, 2001), neurological abnormalities (Datta-Bhutada et al. 1998), problems with later developmental abilities, for example, motor development, verbal skills and memory (Eyler et al. 1998), (Swanson et al. 1999), (Feng, 1993); and poor educational achievement (Eriksson et al. 2000) may result. However, accurate estimates of the size of the risk for many of these outcomes are not available.

3.6 Summary

Recreational drug use is now a major global problem. National data concerning recreational drug use is collected as part of the British Crime Survey. Self-report data from the BCS indicate that the prevalence of the use of recreational drugs increases steadily from age 11, peaks at 20 to 24 years of age and then declines fairly rapidly with increasing age. Other findings indicate that males are more likely to participate in recreational drug use and that the highest prevalence of recreational drug use is found in those of mixed ethnic background and also affluent urban or inner city dwellers. There is very little data available about the prevalence of recreational drug use in pregnant women for the UK, Europe or the USA. Major consequences of recreational drug use include an increased risk of death and health problems. Studies of recreational drug use during pregnancy have shown increased risks of mortality and a wide range of morbidities including congenital anomalies thought to result from vascular disruptions (eg. gastroschisis and small intestinal atresia).

Introduction: Part Four – Hair Analysis for the Detection of Drug Misuse During Pregnancy

4.1 Background

The first reported use of hair analysis for the detection of drug use was in 1954 by Goldblum and colleagues (1954) who were carrying out studies into drug induced dermatitis. However, it was not until 1979 that hair analysis was suggested for the analysis of drugs of abuse, firstly, opiate abuse, (Baumgartner et al. 1979) rapidly followed by phencyclidine (Baumgartner et al. 1981) and cocaine (Baumgartner et al. 1982). Since these initial developments there has been a rapid expansion in this area and a wide spectrum of recreational drugs can now be detected in human hair. These include opiates, heroin/morphine, amphetamine, cannabis, cocaine and LSD as well as a number of the metabolites arising from the drugs (Braithwaite et al. 1995), (Sachs and Raff, 1993), (Nakahara, 1995), (Goldberger et al. 1998), (Segura et al. 1999), (Polettini et al. 1993), (Rohrich et al. 2000a), (Simpson et al. 1997).

Hair analysis for the detection of drugs of abuse has a variety of uses including drug monitoring and control, the determination of the presence of drugs of abuse for forensic purposes, occupational screening for health and safety purposes, assessment of accuracy from self-reported drug use and the determination of prenatal exposure to drugs of abuse. This method of analysis is now used widely around the world (Karacic et al. 2002), (Jurado et al. 1996), (Baumgartner et al. 1989), (Bourland et al. 2000), (Chiarotti et al. 1996), (George and Braithwaite, 1997), (Kikura et al. 1997), (Uematsu, 1993), (Marsh and Evans, 1994) (Marsh et al. 1995), (Mieczkowski et al. 1997), (Simpson et al. 1997), (Nakahara et al. 1997). Nevertheless, there are still analytical issues and ethical dilemmas to be sorted and other areas where this method could be of use, for example in the

testing of drugs in sport, are reticent about using this approach, and continue to use, primarily, urinary analysis.

A major limitation of urinary analysis is, however, that the testing for any drugs is limited to consumption in the previous three days. In addition there are a number of evasive techniques and problems associated with urinary analysis that can be overcome by hair analysis (Baumgartner et al. 1989). Although technical false positive results can be isolated in urinary analysis, contamination or mix up of specimens are not picked up by validation methods. These are easier to identify in hair analysis due to the difference in samples, for example, hair colour and texture. A number of evasive manoeuvres have been used in urinary analysis, despite the closely supervised collection of samples, to ensure a false negative result including the switching of samples, adulteration of a sample with altering substances such as bleach, salt or liquid soap; diluting the sample with water externally or drinking copious amounts of water. Clearly these are problems associated with the behaviour of subjects who wish to evade a positive test result. In the realm of the detection of drug misuse, an illegal activity, this could well be the majority of subjects.

These problems are either minimised in hair analysis or do not exist. Hair can be collected easily without embarrassment, requires no special storage arrangements and is not subject to evasive strategies. Abstinence for a few days will make no difference to the hair analysis results. Hair samples are thoroughly cleaned before analysis to remove any external contaminant. If rigid protocols for the removal of external contaminants are followed, even high levels of external exposure, for example in narcotics officers (regularly handling cocaine amongst other substances), are unlikely to test hair positive (Mieczkowski, 1997). However, in an extreme example of cocaine being rubbed into individual hairs that are then tested for the presence of the drug or a metabolite, false positive results may result (Romano et al. 2001). False positive tests for opiates resulting from the ingestion of poppy seeds are not found in hair analysis whereas in urinary analysis false positive results are produced (Baumgartner et al. 1989).

Unlike urinary analysis, hair analysis offers a wide window of detection of drugs of abuse ranging from months to years, distinguishing between chronic use and inadvertent exposures. Depending upon the length of hair available, this method provides a retrospective history of the timing and severity of drug use that can be separated by the analysis of different segments of hair. A comparison of the main differences between urinary and hair analysis is given in Figure 4.1.

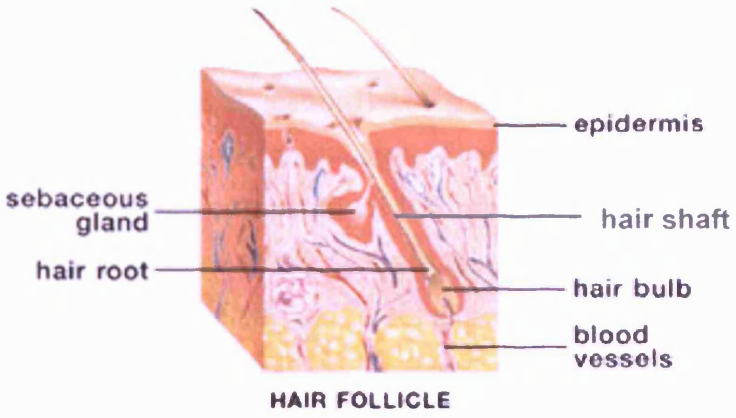
Figure 4.1 Comparison of hair and urine analysis as analytical media for drug testing, from Marsh 1997, (Marsh, 1997).

Hair Sample	Urine Sample
Detects long term drug use (length of hair)	Detects mainly short-term drug use
Easy collection	Requires collection guidelines
Easy storage at room temperature	Storage requires refrigeration or freezing
Identify by colour and texture	Can be exchanged or substituted
Difficult to adulterate sample	Adulteration easier both chemically & biologically
Possible to re-collect comparative sample	Re-collection not comparable to original
Low risk of infection transmission	High risk of infection transmission
Labour intensive relatively costly assay	Automated and cost effective assay
Variable methods	Established methods
Arbitrary cut-off values	Arbitrary cut-off levels
Difficult quality control	Established quality control
External quality assurance not established	Established external quality assurance
Small sample batches desirable	Large sample batches routine

4.2 Hair structure, growth and collection for analysis

Hair is a keratinous protein comprising two main structures: the root or bulb which is buried within the hair follicle in the skin and the exposed shaft. It is postulated that drugs are transferred from the blood in the capillary network that supplies the follicles, into the growing cells of the hair in the root (Cone, 1996). The drugs become incorporated in the hair root and gradually move into the shaft as the hair grows (see figure 4.2). This is thought to occur by passive diffusion from the blood

Figure 4.2: Diagram of hair follicle indicating the blood vessels which supply the hair root (bulb).



Source: Adapted from http://www.keratin.com/aa/aa007.shtml

into the cells in the hair follicle. Other theories include the active diffusion from the blood into the cells in the hair follicle or via body secretions such as sweat and sebum during the formation of the hair shaft. External contamination during hair shaft formation has also been postulated (Wennig, 2000). When hair growth has completed no new drugs are incorporated into the hair. Once bound in the hair matrix drugs are resistant to removal by ordinary daily hygiene activity. Indeed, even specialised washing solutions advertised for "the removal of medications, chemical build up and other unwanted impurities in the hair shaft" fail to completely remove drugs of abuse from the hair leaving detectable amounts in the hair shaft (Rohrich et al. 2000b). Similarly hair preparations such as, hair dye, bleach and perming solutions reduce the amount of the drug in the hair but the drug still remains detectable (Welch et al. 1993), (Marsh et al. 1995). Evidence of the long-term stability of these drugs within the hair once bound into the matrix comes from the detection of cocaine in the hair of pre-Columbian and Egyptian mummies (Cartmell et al. 1991), (Balabanova and Wof, 1989) and post-mortem findings of arsenic in the hair of Napoleon Bonaparte and laudanum in the hair of the romantic poet Keats, (Smith et al. 1962), (Lyon, 1986).

In humans, body hair grows at different rates across the body and this growth rate also varies between the sexes, by age and between ethnic groups. However, hair growing in the posterior vertex region of the scalp shows little variation in the rate of growth between these groups and is therefore the optimal area for collection and analysis. The estimated rate of hair growth in this area is one centimetre per month, (Saitoh et al. Oxford) with a range of between 0.8 and 1.3 centimetres per month in the general population. The suggested procedure for the collection of hair is to select approximately 100 hairs in the posterior vertex region of the head (aesthetically from an area where this will be unnoticeable) and to cut off this group of hairs as close to the scalp as possible. This sample should then be tied at the scalp end of the sample so that the most recent growth can be identified. Other sites of hair growth may be considered for analysis, eg. beard, axillary or pubic hair, if scalp hair is unavailable, however variations in growth and issues of the collection of this hair need to be considered. Baby hair has also been analysed to detect gestational drug misuse in the last two trimesters of pregnancy, (Strano-Rossi et al. 1996), (Marques, 1996).

4.3 Hair growth and pregnancy

In simple terms hair growth, on the scalp, occurs in two main phases. The anagen or growth phase of the hair lasts for several years. This is followed by the telogen or resting phase which lasts for a few months before depilation when the hair is shed, (Lynfield, 1960). Each hair on the scalp has its own individual cycle that is not influenced by the surrounding hairs, so that neighbouring hairs are more often than not at different phases of growth. In the general population the hair in the posterior vertex region of the scalp is approximately 85% anagen phase and 15% telogen phase and therefore within any sample collected for analysis a proportion of the hairs will be in the telogen or resting phase. This can cause problems with timed segment hair analysis. During pregnancy the conversion of hairs from the anagen phase to the telogen phase is slowed down resulting in the majority of the scalp hair being in the anagen phase, (Lynfield, 1960), giving the appearance of increased hair thickness due to the increased density of anagen hairs. This aligning of the phases of hair growth facilitates the timed segment hair analysis of

pregnant women. Following delivery the reverse occurs and the conversion from anagen to telogen phase is accelerated leading to increased hair loss, (Van Scott, 1958). It is postulated that the changes in the hair cycle are due to hormonal influences possible oestrogens (Lynfield, 1960). Contrary to expectation, the rate of hair growth during pregnancy appears to be slightly slower than in the general population at approximately 0.9 centimetres per month, (Pecoraro et al. 1969).

4.4 Methods of Hair Analysis

The most common methods used for analysing hair for recreational drugs are Radioimmunoassay (RIA), Gas Chromatography Mass Spectrometry (GCMS), and Liquid Chromatography tandem Mass Spectometry (LC-MS/MS). Whichever method is used detailed protocols are followed. These include the washing and grinding of the hair followed by the extraction, elution and derivatisation of the drugs. Solutions left over from the hair washing are kept as a double check for contamination if a positive drug result is found. A number of different processes may be used to extract the drugs from the hair which include solvent elution, enzyme digestion, and acid and alkali washing, (Kintz and Cirimele, 1997). Further extraction processes, liquid-liquid extraction or solid phase extraction, are then carried out which help to purify the sample before analysis (Chiarotti, 1993). At all stages it is important to ensure that any extraction procedure does not lead to any (or at least any immeasurable) chemical changes in or loss of the drugs and their metabolites of interest.

Hair analysis methods need to be sensitive enough to be able to detect very small amounts of the drugs of interest with results being measured in ng/mL. Radio-immunoassay techniques can lead to false positive results due to the cross reactivity of antibodies used in the method with other compounds such as cold remedies and anti-psychotic drugs. RIA techniques are therefore often used just as an initial screen for the drugs of abuse. Whilst the sensitivity of RIA has been reported at 92% (Kline at al. 1997), the specificity of this test and other methods of hair analysis are harder to calculate as self-reported data cannot be assumed to be completely reliable and there is no other 'gold-standard' available. However,

specificities of up to 90% have been quoted (Spiehler, 2000). GCMS (a two stage method where molecules are separated according to their level of volatility and then further divided by their electrical characteristics) or LC-MS/MS (a newer, expensive but more precise technique) may then be used after RIA screening to validate any positive RIA findings, as these methods of analysis have a higher sensitivity and specificity then RIA techniques (Kintz, 1996), (Moller et al. 1992), (Jurado et al. 1996), (Pichini et al. 1999). For additional validation of the analysis internal standards are used in each GCMS or LC-MS/MS run. In many studies GCMS and LC-MS/MS are used as the 'gold-standard' for comparisons as sensitivities of up to100% for a variety of the most common drugs (including cocaine, cannabis and opiates) have been reported (Moeller at al. 1992), (Ostrea et al. 2001), (Welp et al. 2003). These studies have, however, been carried out on samples derived from known drug users and not on general populations. As with the RIA hair analysis very few studies attempt to calculate the specificity of the test due to the lack of any comparative 'gold-standard'. Comparisons have been made with urinary analysis results using GCMS but this is problematical as urinary analysis only picks up recent drug use within the past three days. Hair analysis identifies more long-term drug use and drug metabolites are not deposited in the hair until approximately one week after consumption (Kline et al. 1997). Nevertheless, work by Ostrea and colleagues (2001) showed a GCMS specificity of 80% for opiates and 87% for cocaine, based on 58 high risk pregnant women, and suggested that the false positive results could be due to passive exposure or contamination, highlighting the need for an efficient and thorough decontamination process prior to analysis.

4.5 Interpretation of Results

The interpretation of any quantitative results from hair analysis is complex and has led to a number of unanswered questions. The Society of Hair Testing has recognised the need for a consensus on hair testing for drugs of abuse and/or doping agents, (Sachs et al. 1997). A major cause for interest in this area is the testing of hair for banned substances in sport, for example by the International Olympic Committee, who have not, as yet, accepted hair analysis for this purpose.

However, these issues are also of importance in hair analysis for drugs of abuse. The five critical questions are listed in Figure 4.3.

Figure 4.3: Five critical questions concerning hair testing for drugs of abuse / doping agents from Kintz and colleagues (Kintz et al. 2000)

1.	What is the minimal amount of drug detectable in hair after administration?
2.	What is the relationship between the amount of the drug used and the concentration of the drug or its metabolites in hair?
3.	What is the influence of hair colour?
4.	Is there any racial bias in hair testing?
5.	What is the influence of cosmetics treatments?

Threshold doses of drugs of abuse have been evaluated in a number of studies over recent years. As little as 25mg of intravenous cocaine (an average street dose being one gram of powder although the level of purity is seldom known) can be detected for up to six months once it has been incorporated into the hair (Henderson et al. 1996). Other work has found that a single oral dose of 60mg codeine can be detected after eight weeks, (Rollins et al. 1996). Suggested 'cut-off' values for a number of drugs of abuse and their metabolites are presented in Figure 4.4 (Kintz, 2004). In the table 6-monoacetylmorphine (6-MAM) is a metabolite of heroin and THC-COOH is a metabolite of cannabis.

The values in Figure 4.4 are, however, only suggested values and there are other factors that need to be taken into consideration, for example, the presence of codeine in a sample could result from a number of scenarios. Heroin samples always contain codeine and therefore the presence of codeine could indicate heroin abuse. Conversely, as morphine is a metabolite of codeine it can be detected when codeine is abused. In order to differentiate between heroin and codeine abuse it is therefore necessary to take into account the concentration of the drugs involved (Sachs and Arnold, 1989). If the concentration of morphine is higher than codeine then heroin or morphine abuse is likely. Similarly, codeine ingestion is more likely if the concentration of codeine is higher than morphine.

The identification of the 6-MAM metabolite of heroin can help clarify any uncertainty, (Nakahara et al. 1992). If GCMS analysis is carried out in the 'Selected Ion Mode' (SIM mode) then the appropriate ions (usually three) in association with the appropriate retention time for a particular drug can be used to indicate a positive result, validated against the drug standards for each run. Where this is in conjunction with other drug analysis or reported drug use positive tests for both tests or the test and report add an extra level of validation (Wu et al. 1999).

Figure 4.4 Proposed 'cut-off' values for specified drugs of abuse – modified from Kintz (2004,) (Kintz, 2004)

Compound	Cut off (ng/mg)
6-Monoacetylmorphine	0.5
Cocaine	0.5
THC-COOH	0.05
Amphetamines	0.5

Dose-response relationships have been studied using hair analysis to determine whether a subject is a chronic drug abuser or infrequent user. In a judicial study levels of drugs and their metabolites were compared between known regular users who declared different levels of use (Pepin and Gaillard, 1997). Although this work proposed ranges of values for low, medium and high use of heroin and cocaine the authors acknowledged the need for some validation of these values from the self-reported use of the subjects. This, of course, relies on the accuracy of the reporting. Other factors that can affect the dose-response relationship are the purity of the drug consumed and the variation in the uptake of the drug from the blood between individuals (Kintz, 2004). As such, the interpretation of results from hair analysis is still uncertain with respect to the relationship between the amount and frequency of use and the quantitative results from hair analysis.

The amount of the various drugs of abuse that are incorporated into the structure of the hair appears to be related to the melanin pigment in the hair; the more

melanin the more drug incorporated into the hair (Nakahara, 1995). Work has been carried out showing that organic compounds are preferentially incorporated into dark hair (Cone, 1996) confirming this association. Further investigations have concluded that the relationship between the melanin content of hair and the rate of incorporation of codeine is exponential (Kronstrand et al. 1999). Higher rates of drug incorporation into, for example, black hair compared to blonde hair, will clearly have an effect on the amount of the drug found during hair analysis and the lower threshold for subject with varying hair colour. Logically this translates into racial variations due to hair colour, where an additional factor affecting the incorporation of drugs into the hair is hair texture (Henderson et al. 1998), (Cone, 1996). These studies have suggested that coarse dark hair may incorporate drugs more readily than fine brown or blonde hair, a factor that should be taken into consideration when interpreting the results of hair analysis.

Cosmetic treatments such as perming solutions, bleach and dyes, affect the stability of drugs in the hair shaft thus reducing their concentration. This may lead to false negative results in some subjects, particularly in those who are infrequent drug users (Jurado et al. 1997), (Potsch and Skopp, 1996). In these circumstances other sites of hair collection could be used to validate findings, although this may not be acceptable to study subjects. False positive results from the external exposure of cosmetic treatments containing a variety of drugs have been found to be very rare (Cirimele et al. 1999), (Pragst et al. 1998). As such it has been concluded that in hair analysis a false negative result is more likely than a false positive result (Cirimele et al. 1999). With strict adherence to protocols and highly sensitive GCMS techniques these false positives can be kept to a minimum.

Hair analysis using either GCMS or LC-MS/MS is the current method of choice for determining an individual's drug abuse status. It is a very powerful tool that with tandem mass spectrometry has recently demonstrated an ability to detect a 'date rape' drug (gamma-hydroxybutyric acid or GHB) following a single exposure to the drug (Kintz et al. 2003). Indeed, positive evidence of drug abuse has been found from the analysis of a single hair, (Wainhaus et al. 1998).

4.6 Summary

Hair analysis is now regularly used to detect the presence of recreational drugs (or their metabolites) in human hair. The major advantage of hair analysis over urinary analysis is the wide window of detection of these drugs, from weeks to months compared to the most recent three days. As a result hair analysis has the ability to distinguish between single and chronic drug abuse and can give an indication of the timing of consumption, given an adequate length of hair. It is therefore an appropriate method to use for the validation of retrospectively collected data concerning recreational drug use during pregnancy following the delivery.

Hypothesis and Study Objectives

5.1 Background

In the late 1990's a number of neonatologists in the former Trent health region noticed that there seemed to be an increase in the number of infants with gastroschisis admitted to their neonatal intensive care units. Investigation of these local cases suggested that more cases seemed to be occurring and that most of the mothers of these cases were under the age of 25 years. A review of the literature confirmed similar experiences around the world over the past two decades or so.

Recreational drug use has also increased substantially over the same period, particularly in younger people. The prevalence of recreational drug use during pregnancy and the subsequent impact upon the fetuses of pregnant women, within this group is, however, unknown. Research suggested an increased morbidity in this group, with an increased prevalence of growth restricted infants and a possible associated increase in the prevalence of gastroschisis was postulated.

5.2 The Hypothesis

This project was designed to test the hypothesis that the risk of gastroschisis is positively associated with the use of recreational drugs in the weeks immediately following conception.

5.3 Objectives

The objectives of this study were to:

(i) To estimate the odds of gastroschisis associated with the use of recreational drugs in the first trimester of pregnancy.

(ii) To validate data collected at maternal interview concerning recreational drug use during pregnancy using maternal hair analysis.

(iii) To estimate the overall prevalence and birth prevalence of gastroschisis in the three health regions: Trent, West Midlands and Northern, over the period 1st January 2001 to 31st August 2003.

(iv) To estimate the population attributable risk of gastroschisis associated with recreational drug use in the first trimester of pregnancy.

Methods

6.1 Introduction

Gastroschisis is a rare anomaly with a birth prevalence of between 2 and 4 per 10,000 births (www.eurocat.ulster.ac.uk). The idea for this study originated from the Trent Congenital Anomalies Register. However, due to the low prevalence of gastroschisis a large birth cohort was required to ensure that there would be sufficient cases for the study to have adequate power. A collaborative research project was therefore established between three geographically defined regional congenital anomalies registers: the Trent Congenital Anomalies Register, the West Midlands Congenital Anomalies Register and the Northern Congenital Abnormality Survey. It was estimated that together these three registers would provide a population of approximately 157,500 births per annum: that is, over one-quarter of the total births for England.

The investigation reported in this thesis was designed as an aetiological, matched case control study to test the hypothesis that the risk of gastroschisis is positively associated with the use of recreational drugs in the weeks immediately following conception. The eligible study population was all Trent, West Midlands or Northern region resident mothers, who delivered a fetus or infant with gastroschisis during the period 1st January 2001 to 31st August 2003.

The principal investigator (ESD) for the study secured funding to allow each region to employ a part- time research midwife or research health visitor to carry out the data collection. These will be referred to as 'the interviewers' throughout this thesis. A Project Management Group was established comprising the project principal investigator, representatives from each regional register and the interviewers to ensure the smooth running of the study. This group met twice yearly over the data collection period.

6.2 Study Design

A case control study design was selected as the most appropriate method for the investigation of the aetiology of rare conditions. The study population was all deliveries and terminations of pregnancy to mothers resident in the geographical area of the three collaborating regional Congenital Anomalies Registers over the period January 2001 to August 2003. The cases were defined as the mothers of all gastroschisis affected pregnancies (ICD 10 code Q79.3), resident in the three geographically defined areas covered by the congenital anomalies registers. Three mothers of live born infants, not affected by gastroschisis, were matched to each case by maternal age to within one year, initial intended place of delivery and region of maternal residence. All case and control mothers were interviewed within six weeks of delivery. Data were also abstracted from antenatal and obstetric notes. Maternal hair samples were collected to objectively measure recreational drug use and validate maternal report.

The three regional registers were used to notify the study personnel of gastroschisis cases, as soon as possible, following their antenatal or subsequent detection and notification.

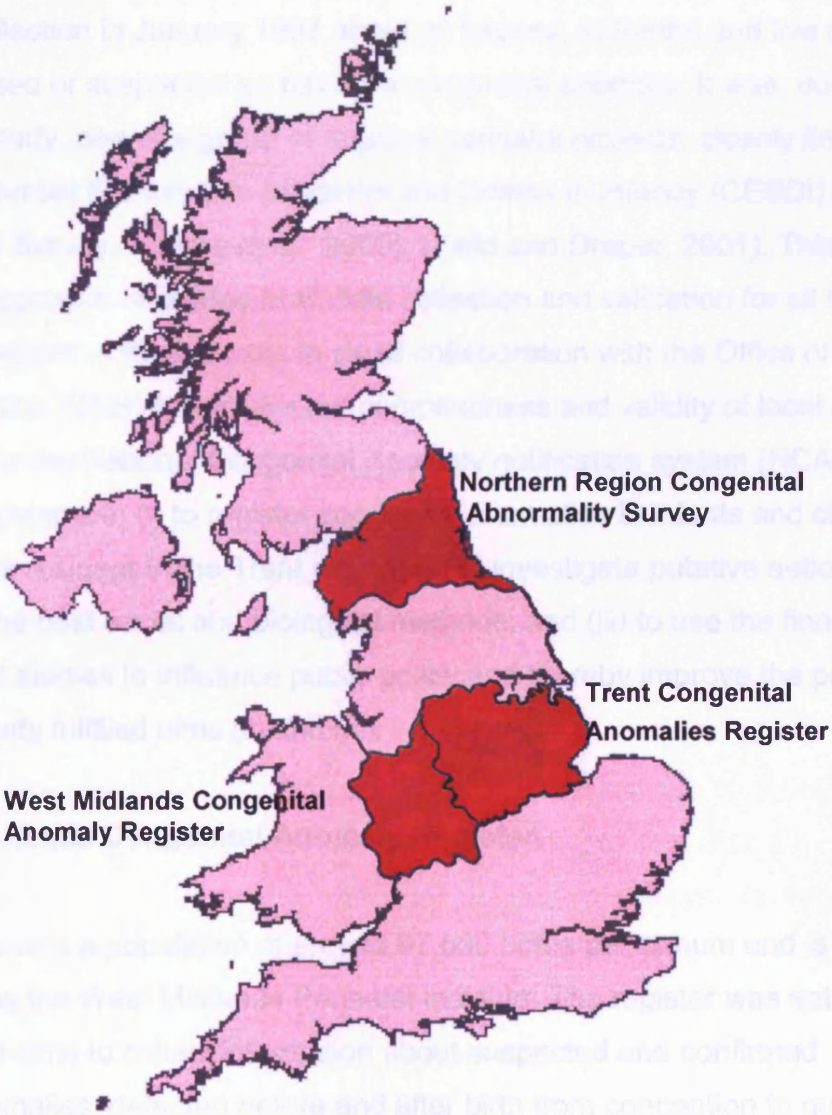
6.3 Study Sample size and power

Assuming a gastroschisis prevalence of between 2.8 and 3 per 10,000 births (based on the most recent data from the Trent, West Midlands and Northern Registers), 157,500 births per annum and a period of data collection of two years and 6 months, it was estimated that between 110 and 118 cases of gastroschisis would be available from the three registers. With this number of cases it was estimated that, assuming an exposure to recreational drugs in the control group of 10% (Ramsay et al. 2001), in order to detect an odds ratio of 2.5 at the 5% level of statistical significance, with 80% power, a ratio of three controls for every case would be required.

6.4 Description of the Collaborating Congenital Anomalies Registers

This study was developed before the most recent NHS re-organisation on 1st April 2002. The descriptions of the regional registers are therefore based on the boundaries prior to April 2002.

Figure 6.1: UK map indicating the geographical location of the three collaborating regional congenital anomaly registers



All three regional congenital anomaly registers encourage multiple source notification and have developed large multidisciplinary networks of health professionals who complete notification forms. Data sources include: ultrasound and radiology departments, genetics departments and cyto-genetics laboratories, neonatal screening visits, antenatal clinics, delivery suites, pathology laboratories, paediatric surgery departments and neonatal, paediatric, obstetric and gynaecology departments.

6.4.1 Trent Congenital Anomalies Register (Trent CAR).

This register covers a population of approximately 60,000 births per annum and began data collection in January 1997 about all fetuses, stillbirths and live born infants diagnosed or suspected as having a congenital anomaly. It was, during the period of this study, one of a group of regional perinatal projects, closely linked with the Confidential Enquiry into Stillbirths and Deaths in Infancy (CESDI) and the Trent Neonatal Survey, (Clarke et al. 2000), (Field and Draper, 2001). This collaborative approach facilitated both data collection and validation for all these studies. The register staff also work in close collaboration with the Office of National Statistics (ONS) to improve the completeness and validity of local and national data for the National Congenital Anomaly notification system (NCAS). The aims of the register are: (i) to register congenital anomalies in infants and children born to mothers resident in the Trent region; (ii) to investigate putative aetiological factors using the best social and biological methods; and (iii) to use the findings of the aetiological studies to influence public policy and thereby improve the public health. This study fulfilled aims (ii) and (iii).

6.4.2 West Midlands Congenital Anomaly Register.

This register covers a population of around 67,500 births per annum and is administered by the West Midlands Perinatal Institute. The register was set up in June 1994 and aims to collect information about suspected and confirmed congenital anomalies, detected before and after birth from conception to outcome, up to the first 2 years of life, i.e. all late fetal losses, terminations of pregnancy, still

and live births. The Congenital Anomaly Register is maintained on the same database as the register of West Midlands CESDI notifications of fetal and infant deaths providing an additional level of validation between these two data sources.

6.4.3 Northern Region Congenital Abnormality Survey (NorCAS).

This survey covers a population of about 30,000 births per annum, the geographical boundaries of which are defined by the old pre 1994 NHS regional boundaries. It has been in operation since 1984 and collects data about all major congenital abnormalities in fetuses, stillbirths and live born infants of mothers resident in the Northern region. NorCAS is located within the Maternity Surveys Office of the Northern region, thus allowing for data validation with Northern CESDI, the Regional Perinatal Mortality Survey and the Multiple Births Register (Atkins and Hey, 1991), (Northern Regional Survey Steering Group, 1992). A regular exchange of data is also carried out with ONS.

6.5 Data collection

A structured questionnaire was developed for this study to collect information from case-note review and maternal interviews. In addition, a sample of maternal hair was collected during the interview with the mother. This was analysed to objectively measure maternal recreational drug use and validate reported recreational drug use in early pregnancy. A copy of the questionnaire can be found in Appendix A.

The questionnaire was divided into two sections. Section one was completed from a review of the maternal case notes plus any other relevant antenatal information and information from post mortem reports, as appropriate. Any missing information from the antenatal period was requested from the mother's General Practitioner. A copy of the discharge report was requested for cases where the infant had been cared for in a neonatal intensive care unit or paediatric surgical unit. Data items collected in this section are listed below:

Study identification number and region of residence
Postcode, health district of residence
Obstetric consultant, place of delivery, initial intended place of delivery (at booking), intended place of delivery (at the onset of labour) and actual place of delivery
Case/control identifier
Maternal date of birth and marital/cohabitation status
Maternal height and pre-pregnancy weight (collected to calculate body mass index: BMI=weight(kg) / height(m)^2)
Family history of congenital anomalies
Previous pregnancies/births including any affected by congenital anomalies
Mother's general and present medical history
Antenatal care details including LMP, EDD, gestation at first recorded contact
AFP testing and results
Fetal anomaly scan and X-ray results
Delivery gestation, mode and type of labour, fetal distress as described by the accoucheur/se
Infant details, date and time of birth, birth weight, sex, apgar scores
Details of the date and cause of death where applicable
Presence of congenital anomalies in the index pregnancy

Section two was completed from an interview with the mother, carried out, with consent, within six weeks of the expected date of delivery. The interview comprised questions about:

GP and surgery details
Maternal occupation, working conditions, exposure to chemicals, use of equipment
Paternal occupation, working conditions, exposure to chemicals, use of equipment
Details of prescribed or 'over the counter' medicines and period of use during pregnancy
Use of recreational drugs and period of use during pregnancy
Ethnic group and religion
Smoking status: ever, period of use during pregnancy
Alcohol consumption: ever, during pregnancy (consumption during the first trimester and the month before delivery), type of alcohol and frequency of consumption, bingeing
Caffeine consumption during pregnancy
Consanguinity in this pregnancy
Census derived questions: house and car ownership
Use of hair preparations

Prompt cards (see Appendix B) were used to help with the questions relating to any prescribed, 'over the counter' or recreational drug use during pregnancy. In the case of prescribed or 'over the counter' medicines the interviewers ran through a list of categories of drugs, checking whether the mother had had an illness

during her pregnancy that would require such medication and asking to view any medicines, tablets or ointments that the mother had taken (if available). A comprehensive list of the different types of recreational drugs, that might have been taken, was then read to the mother asking if she had taken or used any of these substances during her pregnancy. Additional prompt cards, indicating the 'street' names for the different drugs, were also provided for the interviewer to use if required. The information about street names was downloaded from the internet. Details of the use of any hair treatments and the ethnicity of the women were recorded as these are known to reduce the concentration of any recreational drugs and their metabolites within the hair (Cone, 1996), (Potsch and Skopp, 1996), (Pragst et al. 1998).

Finally, if permission was granted, a sample of approximately 100 maternal head hairs was collected from the posterior vertex region of the scalp. These samples were tied together at the scalp end of the hair, sealed in a plastic bag and marked with details of the study identifier, date of the last menstrual period, the expected date of delivery and the date of sample collection.

6.6 Pilot studies

6.6.1 Questionnaire

The study questionnaire was adapted from the instrument used for the Leicestershire Perinatal Mortality Survey (Draper et al. 1999). Following the removal of any irrelevant questions from this instrument a number of new questions were developed to collect information concerning recreational drug use during pregnancy, exposure to chemical and equipment related to hobbies, caffeine consumption and the use of hair preparations. Questions concerning maternal height and pre-pregnancy weight were also added in order to calculate the body mass index for the mothers. In order to test the comprehensibility and face validity of the new questions six female members of staff within the

Department of Epidemiology and Public Health, University of Leicester were interviewed. They reported no difficulty in interpreting any of the questions.

6.6.2 Hair length

The rate of hair growth during pregnancy is estimated to be approximately 0.9 cms per month (Pecoraro et al. 1969). Allowing for some variation in this rate in the pregnant population it was estimated that to carry out hair analysis to detect recreational drug use at around the time of conception it would be necessary for the women to have hair of at least nine centimetres in length. A small pilot study of all young women, under the age of 25 years, attending on one morning in May 1999 for their antenatal appointment or as an in-patient at the University Hospitals of Leicester NHS Trust, Leicester Royal Infirmary Maternity Unit, showed that out of a possible 12 women, 11 had hair of over nine centimetres in length.

6.6.3 Collection of hair samples

An important feature of this study was to objectively measure exposure to recreational drugs in pregnancy and to validate the data relating to recreational drug use, collected by maternal report, by analysing a sample of maternal hair. It was therefore necessary to establish the feasibility of the collection of a hair sample from a group of mothers. In a small pilot study, carried out on two separate days in June 1999, all women aged 20 years or less were approached on the postnatal wards of the two main maternity units of the University Hospitals of Leicester NHS Trust. All 11 women gave permission for a small sample of their hair to be taken for analysis following the receipt of an information sheet. These women also agreed to answer questions concerning their use of hair preparations and treatments. This pilot concentrated on very young mothers as it was anticipated that a large proportion of gastroschisis case mothers would fall into this category. The prevalence of recreational drug use was also anticipated to be higher within this group. As such it was necessary to investigate whether, in general, they would be willing to participate in this type of study.

6.7 Interviewer training and monitoring

Three interviewers were employed for this study: one from each region. All interviewers had a background in midwifery, health visiting or both specialties. Training sessions were held with all the interviewers covering the principles of interviewing. These included interviewing techniques, the rationale and measures used to achieve standardisation of data collection and inter/intra observer variability. The interviewers were then given time to familiarize themselves with the questionnaire, the layout and the use of the suggested prompts and the flash cards. Video recordings of practice interviews were reviewed and feedback sessions were provided. The interviewers were then accompanied on a number of study interviews to ensure that high standards were maintained. Annual review sessions were then held within the Project Management Group so that the style and standardisation of data collection could be assessed and monitored. Individual interviewers were observed on visits at various stages during the study.

6.8 Selection and Recruitment of subjects

6.8.1 Case definition and selection

All gastroschisis affected pregnancies, coded Q79.3 by ICD10, to mothers resident in of one of the three participating regions, were eligible for inclusion within the study. As soon as a new case of gastroschisis was notified to one of the registers this information was fed through to the study personnel. To ensure the complete and prompt ascertainment of all cases a network of clinical contacts was established in all units around each region. The interviewers regularly contacted the fetal medicine, neonatal and paediatric surgery units to check for any newly diagnosed cases or admissions. Confirmation of the diagnosis of a gastroschisis case was checked with the relevant clinicians and against post mortem reports, where appropriate, prior to inclusion. Body wall complex and body stalk anomalies were excluded. In rare cases a gastroschisis is thought to result from a mechanical rupture of the umbilical membrane near the time of birth. Such cases were identified at post mortem and excluded as this perinatal variant appears to have a completely different aetiology (Shaw, 1975).

6.8.2 Control definition and selection

Three live birth controls were selected for each case of gastroschisis. These controls were defined as three subsequent live born infants delivering in the initial intended place of delivery of a gastroschisis case, matched by region and maternal age (to within one year). The justification for the use of 'normal controls' and not 'malformed controls' is discussed in chapter 11, section 11.1.3. The control subjects selected for cases that were terminations of pregnancy were selected following the expected date of delivery of the case. Control subjects who refused to participate in the study were replaced using the next available, appropriately matched mother of a live born infant.

6.8.3 Recruitment of gastroschisis case mothers

The method of approach to mothers in perinatal research in general is highly sensitive and forms an integral part of the ethics approval process. In order to establish a method for approaching the women for this study a letter was written to all obstetricians, neonatologists and paediatric surgeons providing care for pregnant women and their infants in the three regions asking for their support for the study. A copy of the study abstract and contact information for the study was included in the letter (copy in Appendix C). Presentations about the study were also given at a variety of educational days for clinicians and other health professionals in the three regions. In this way a network of relevant clinicians was created for the study building upon the network already established for the congenital anomalies registers.

Data from the Registers showed that the majority of gastroschisis cases are detected during the mid-trimester of pregnancy and notified at that stage to the relevant congenital anomalies register. This facilitated the recruitment of the cases to the study. Contact was made with the mother's obstetric consultant in these cases, asking them to provide information to the mothers about the study and asking them to seek the mother's permission (at around 36 weeks gestation) to allow the study interviewer to contact them following delivery. This allowed the recruitment process to take place after mothers had had a period of time to

discuss diagnosis, possible causes, outcome and treatment of the anomaly with their consultant. Mothers were then contacted either personally, whilst in hospital, or by letter following discharge. In cases where notification of the anomaly did not occur until after delivery or termination of pregnancy all initial contact was carried out by the mother's obstetric consultant or via the neonatal or paediatric clinician prior to any approach by the interviewers. In the rare circumstance of the mother losing contact with the unit an approach was made via the mother's General Practitioner. All recruited case mothers were interviewed within six weeks of their expected data of delivery to minimise any problems with recall.

All mothers were provided with an information sheet describing the study (see Appendix D) and given time to ask questions of the study interviewer and to discuss the study with her family. Mothers were informed that a small hair sample would be collected to validate the information they provided at interview about substances that they might have been exposed to during pregnancy. A requirement of MREC approval was that prescribed medications and recreational drugs were explicitly mentioned. When a mother was satisfied that she had received adequate information and had decided that she wished to participate in the study arrangements were made for a mutually agreeable appointment. A consent form (see Appendix E) was completed prior to the interview whilst ensuring that the mother understood the purpose of the study and her involvement. If a mother was unable to understand English then a confidential NHS interpreter was used to facilitate the information provision, consenting and interview process. In circumstances where a mother was under the age of sixteen years this was carried out with both the mother and her parent/guardian as appropriate. If an underage mother was deemed to be competent by the clinical staff to give consent without parental involvement the Trent Multi-centre Regional Ethics Committee had instructed the study personnel to accept the consent of the mother alone.

Mothers were never contacted at home or within the hospital without prior discussion with their obstetric team concerning the mothers' ability to cope with the situation. This method of approach was based on the Leicestershire Perinatal

Mortality Case-Control Study (Draper et al. 1999) which has been running successfully since 1976.

Arrangements were set in place for the interviewers to refer mothers for counselling if this was felt to be required following the study interview. Mothers were also provided with information concerning support groups and networks for either bereaved parents or the parents of infants with an abnormality, as appropriate.

As the occurrence of a case of gastroschisis was a fairly rare event for individual hospitals, within the three regions, the interviewers maintained regular contact to ensure that the study maintained a high profile and cases were not missed. In addition, an annual Christmas card was sent to each contact, thanking them for their support and giving details of the progress of the study and the numbers of mothers recruited within their unit.

6.8.4 Recruitment of matched control mothers

A network of midwives was established across the three regions to assist in the selection and recruitment of control mothers for the study. Following the delivery of a gastroschisis case at a particular maternity unit the relevant midwife from the network was contacted and asked to select three control mothers, matched to the gastroschisis case by the matching criteria. If the selected control mother was still an inpatient at the unit, this midwife then provided her with an information sheet from the study, answered any study related questions and asked for her permission to be approached by the study interviewers. Where a mother had been discharged home her community midwife was contacted and asked to carry out the same recruitment process. For those who agreed the interviewers arranged appointments with the mothers in the same way as for the cases including all the control mothers being interviewed within six weeks of their expected date of delivery to minimise any problems with recall.

6.8.5 Non responders

Limited data was collected about non-responding case mothers. A minimum data set is collected for all notified anomalies to each congenital anomaly register. The Congenital Anomalies Registers have both Multi-centre Research Ethical Committee and Patient Information Advisory Group (class support under section 60 of the Health and Social Care Act 2000) approval for data collection without explicit consent. Anonymous data were provided to the study to allow the description of non-responding gastroschisis cases:

Region of residence
Maternal age
Gestational age at delivery of gastroschisis case
Singleton / multiple pregnancy
Ethnic group of mother
Birth weight and gender of gastroschisis case
Outcome (ie. stillbirth, livebirth etc)
Isolated / multiple anomaly (with details of all other anomalies)
Antenatal detection, yes or no.

Due to patient confidentiality and ethical issues no additional data could be collected about the non-responding controls. Details of the characteristics of these mothers used for the matching process were, however, known.

6.9 Data Handling

6.9.1 Data coding and checking

A coding manual was devised for use with the study questionnaire. A copy of this manual is provided in Appendix F. The three interviewers coded the questionnaires following their completion and collected any outstanding information from neonatal discharge letters, post mortem reports and any additional data required from general practitioners or other hospitals that provided care for the mothers and infants. The questionnaires were then sent into the study office in Leicester where they were double-checked for completeness and any

missing codes were added. The principal investigator then re-checked the completeness and accuracy of the coding of all questionnaires.
Details of the medical history, congenital anomalies and causes of death, where appropriate, were coded using the 10th edition of the International Classification of Diseases (ICD10). Maternal and paternal occupational details were coded using the Office of Populations Censuses and Surveys (now ONS) Classifications of Occupations (OPCS, 1991) and the industries in which they worked were coded using the Classification of Industries (Central Statistical Office, 1995). These versions of the occupational and industrial classifications were used to allow for future comparisons with the control mothers from the Leicestershire Perinatal Mortality Survey (Draper et al. 1999). Prescribed and 'over the counter' medicines used by the mother during pregnancy were coded using the British National Formulary (British Medical Association and Royal Pharmaceutical Society of Great Britain, 2003).

6.9.2 Data entry

An Access database was developed to allow for both data entry and validation of the coded data from the questionnaires. This database required double data entry to ensure the highest level of accuracy. Inconsistencies were identified and rectified on the second data entry. Any inconsistencies arising from the miscoding of the data were then highlighted and checked with the principal investigator.

6.9.3 Data cleaning

Following the completion of the data entry and any additional data checking further data cleaning was carried out using the completed data set. This involved the cross-tabulation of logically related variables and final range checks for individual variables. Any inconsistencies in the data were identified and outliers for specific variables validated using both the questionnaires and any additional data available from the interviewers.

6.10 Hair analysis

6.10.1 Background

During the early development of this study consideration was given to the optimal method of objectively measuring drug exposure and validating data collected at maternal interview. Following attendance at a seminar on the benefits of hair analysis for the detection of recreational drug use by Dr Andrew Marsh (Marsh, 1997), (Marsh et al. 1995), (Marsh and Evans, 1994) the decision was made to use this method in collaboration with the Chemical Pathology Departments, both within the University of Leicester and the University Hospitals of Leicester NHS Trust, who expressed an interest in developing an expertise in hair analysis. In preparation for this project a member of staff from the NHS Trust completed an MSc thesis entitled 'Analysis of Drugs of Abuse in Hair', at the University of Sheffield and Sheffield Hallam University, to establish the required extraction and sample cleansing methodologies within the local laboratory.

6.10.2 Gas chromatography mass spectrometry analysis of all hair samples

All hair samples collected in the study were stored and then sent to the laboratory for analysis in batches of 100 samples in the final year of the study. An Excel spreadsheet was developed for the allocation of random numbers to the hair samples and calculation of the measurements for the segment for hair analysis. Prior to the labelling of the hair samples with random numbers and segment measurements the data were checked by the principal investigator (ESD). All samples were then labelled with a random number (to prevent any bias resulting from the knowledge of the case or control status of the sample), and measurements, in centimetres, indicating the portion of the hair sample that was estimated as having grown during weeks four to nine post conception (the postulated period of the development of gastroschisis (deVries, 1980), (Hoyme et al. 1981)); the segment for analysis. This measurement was revised after the first 200 samples had indicated that this small segment of hair weighed too little, in many instances, and was therefore inadequate for the hair analysis. The

remaining samples of hair were therefore marked with revised measurements indicating the growth during the whole of the first trimester of pregnancy.

Gas Chromatography Mass Spectrometry (GCMS) analysis of the hair was used to detect evidence of recreational drug use. The hair samples were tested for the list of drugs and metabolites listed in Table 6.1. Preparation of the hair samples prior to GCMS analysis commenced with a rigorous washing of each sample to remove any external contaminants followed by grinding of the hair in a ball mill grinder. A process of extraction of any recreational drugs within the hair was then carried out followed by elution and derivitisation of the metabolites of any recreational drugs. A full protocol for the extract, elution and derivitisation process can be found in Appendix G. Analysis of hair washing solutions was also carried out to help in the interpretation and validation of the results. Results were presented as either positive or negative for the various recreational drugs and metabolites.

Table 6.1: List of recreational drugs and metabolites tested for using GCMS

Drug / metabolite	Description	Drug Group
DHC	Dihydrocodeine	Possible Opiate
Morphine	Heroin	Opiate
Codeine		Possible opiate
Methadone	Heroin substitute	Opiate
Amphetamine		1st generation amphetamine
MDMA	3-methoxy-4, 5-methylenedioxamphetamine	Amphetamine derivative (Ecstasy)
6-MAM	6-monoacetylmorphine	Opiate (metabolite of heroin)
Cocaine		Cocaine
Benzoylecgonine	Metabolite of cocaine	Cocaine
TNC COOH	Cannabis	Cannabinoid

The first set of GCMS analyses described here produced a set of results in which there was no overlap between the positive results from maternal interview and the positive results from the hair analysis. The laboratory results book in which all samples were recorded in the laboratory, the Excel spreadsheets of the random number allocation and the results were checked methodically to investigate if a mix-

up of the results had occurred. A number of individual errors were picked up in this way but no evidence could be found of a systematic error. The results of this first GCMS analysis were therefore discarded as inaccurate and a 'rescue-plan' was devised. This is discussed in detail in chapter 11, section 11.2.2.

6.10.3 Radio-immunoassay (RIA) screening and validation by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) for selected hair samples.

In an effort to rescue some results from the GCMS hair analysis a second round of hair analysis was proposed by the Department of Chemical Pathology at the UHL NHS Trust. It was not possible to repeat the analysis on all hair samples. Indeed, some tested hair samples were no longer adequate for analysis as most of the sample had been used in the GCMS analysis. Thus, samples of hair were selected (i) where a positive result for recreational drugs was found by the GCMS analysis, (ii) where a positive result for recreational drugs was anticipated from the maternal interviews and (iii) a small sample (n=15) of hair samples that were negative for recreational drugs from the GCMS analysis and maternal interview data. Hair segments were selected from the remaining hair samples from the portion of the hair adjacent to the first sample i.e. one centimetre before the first trimester hair sample and for the remainder of the pregnancy period. Information about the use of recreational drugs was collected for the whole pregnancy in the maternal interview and therefore this data was used to corroborate any positive findings for recreational drugs from this analysis. Following the preparation of these samples they were screened for the detection of recreational drugs using radio-immunoassay (RIA).

Results were presented as negative, borderline positive or positive from this procedure. Borderline positive and positive results were re-analysed for validation purposes for all drugs, with the exception of cannabinoids for which there is no confirmatory assay available. This re-analysis was carried out using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). Details of the cut-off values used in the detection of the recreational drugs and metabolites for both the RIA and LC-MS/MS are given in Appendix H. As with the RIA results, data from

maternal interviews were compared with any positive findings for recreational drug use.

6.11 Data Analysis

The data were analysed in three stages. First, an overall description of the gastroschisis and control data was carried out. Second, a conditional logistic regression analysis was carried out to test the primary hypothesis. Finally, data from the hair analysis were added into the conditional logistic regression model to test the effect on the study results.

6.11.1 Descriptive analysis of the study data

Using data for all known gastroschisis deliveries over the period of the study the overall prevalence (including late fetal losses and terminations of pregnancy) and the birth prevalence were calculated by year, by region and for the whole study.

Regional response rates were calculated for each region separately and then overall. The characteristics of the responding and non-responding gastroschisis mothers and their infants were compared to identify any significant differences. The formal level of statistical significance was set at $p<0.05$. The Mann Whitney U test was used to test for differences between the responders and non-responders for the continuous variables maternal age, gestational age and birth weight. The chi-squared test was used to test for differences between the responders and non-responders for binary and categorical variables eg. ethnic group and outcome of pregnancy. Fishers exact test was used in instances where there were less than five observations within any one cell.

In this study, three control mothers were matched to each gastroschisis mother, by age (to within one year), initial intended place of delivery and region of residence. The success of matching on maternal age was examined using age as a categorical variable using a chi-squared test and as a continuous variable using a

Mann Whitney U test. The proportions of the control mothers successfully matched by initial intended place of delivery and region of residence were also compared.

A descriptive analysis of the pregnancy care characteristics and details about the labour, delivery and outcome of pregnancy for the gastroschisis mothers and their matched controls was then carried out. Finally, factors, known to or suspected of increasing the risk of gastroschisis were examined. These were socio-economic characteristics, medical history, working conditions and occupational exposures, use of medication and risk behaviours during pregnancy. A mother was defined as being supported (ie. financially) if she was either married or cohabiting with her partner. Body mass index was categorised as underweight, normal, overweight and obese using the following categories: less than 18.5, 18.5 to <25.0, 25.0 to <30.0 and 30.0 or more, respectively (NHLBI, 1998). A new composite variable, gynaecological infection or disease, was constructed for mothers for whom one or more of the following was recorded: recurrent urinary tract infections, chlamydia or an abnormal smear result recorded in their medical history. Binge drinking was defined as the consumption of six or more units of alcohol on a single occasion (Bridgwood, 2000).

6.11.2 The conditional logistic regression analysis.

For all the conditional logistic regression analyses, statistical inferences were made on the basis of estimates of the odds ratio and their 95% confidence interval. Odds ratios were determined to be statistically significant if the 95% confidence interval did not include 1.0. All conditional logistic regression analyses were carried out using STATA v7 (Anonymous2001).

In this study three controls were matched to each case and any non-responding controls were replaced until this ratio was achieved. In order to test whether this control replacement introduce bias, all conditional logistic regression analyses were carried out twice: (i) using all three matched controls per case, and (ii) using only those controls from the initial selection process i.e. without replacement.

These analyses were designed to test the hypothesis that the use of recreational drugs in the weeks following conception is a risk factor for the development of gastroschisis. Three exposures of interest were defined *a priori* using various combinations of recreational drug use: (i) first trimester use of any recreational drug; (ii) first trimester exposure to a class A or B drug; and (iii) first trimester exposure to cannabis only, class A or B drugs only or to both cannabis and class A or B drugs.

Unadjusted odds ratios were calculated using conditional logistic regression analysis for all potential confounding factors from the descriptive analysis. A series of models were fitted, each containing either a single binary indicator variable or the various levels of categorical variables. The baseline comparison group for each variable is presented first.

Socio-demographic risk factors

Parity *(parity 1, primiparous, parity 2 or more)*
Ethnic group *(white, asian, black, mixed/other)*
Marital status *(married, single/separated/divorced)*
Support status *(financially supported, unsupported)*
Overall social class *(I/II/IIInM, IIIM/IV/V, unemployed)*
Mother worked during pregnancy *(no, yes)*
Homeowner *(yes, no)*
Car available for personal use *(yes, no)*
Body mass index *(normal, underweight, overweight, obese)*

First trimester exposure to medications, X-rays or medical history risk factors

Aspirin *(no, yes)*
Ibuprofen *(no, yes)*
Antihistamine *(no, yes)*
Phenylpropanolamine *(no, yes)*
Paracetamol *(no, yes)*
Oral contraceptives *(no, yes)*
Anti-depressant drugs *(no, yes)*
X-rays *(no, yes)*
History of depressive illness *(no, yes)*
Recurrent gynaecological infection/disease *(no, yes)*
Family history of congenital anomalies *(no, yes)*

Risk behaviours during the first trimester

Caffeine consumption *(none,1-9, 10-19, 20+ cups/day)*
Cigarette consumption *(none,1-10, 10+ cigarettes/day)*
Alcohol consumption *(none, <14 units, 14-<28, 28+ units/week)*
Binge drinking *(none, 6-10, 11-20, 20+ units on one occasion)*
Any recreational drug *(no, yes)*
Cannabis *(no, yes)*
Class A or B drugs – namely: heroin, cocaine, amphetamine or ecstasy *(no, yes)*

A stepwise conditional logistic regression analysis, using case control status as its binary response variable, was constructed by stepping down from a main-effects model, including the exposure variable of primary interest (i.e. recreational drug use) and all potential confounding variables as covariates. The order of removal of these variables was determined by the size of the univariate odds ratios. The likelihood ratio chi-squared value corresponding to the change in deviance (and change in degrees of freedom) associated with their removal was observed. Those variables that did not have a statistically significant effect ($p<0.05$) on the fit of the model were removed. Once a final model, containing only those variables that made a significant contribution to the fit of the model had been constructed all possible two-way interaction terms between covariates in the final model were created and added to the model one by one. Statistical significance was assessed using the change in deviance. As this component of the modelling invoked extensive multiple testing, a significance level of $p<0.01$ was used to determine statistical significance.

In order to test the robustness of the final model a number of checks were carried out. First, cases of gastroschisis that were also associated with other anomalies were removed from the model, one at a time, to observe their impact on estimated parameters. Second, observations associated with outlying (excessively high) measures of recreational drug use were also removed: that is, the five highest users of recreational drugs were removed from the model one by one. Finally, the model was checked for the impact of observations with unusual influence or leverage. Neither can be estimated easily in a matched case-control analysis as the 'predict' function in STATA will not generate estimates of the hat matrix or $\delta\beta$ (the change in the estimated value of a log odds ratio of interest associated with

the removal of a particular observation from the analysis). In consequence, the required δβs were generated manually: $\delta\beta_j$ (the change in the log odds ratio associated with the j^{th} observation) was estimated by manually removing the j^{th} observation, refitting the model to re-estimate the required log odds ratio, and then replacing it prior to calculating the next δβ. At the end of this process each observation was associated with an estimated δβ. These were sorted and the observations associated with the five most positive and five most negative δβs were deleted from the model *en bloc*. It was argued that any relationship that still remained could not, by definition, be consequent solely upon extreme observations with excessive influence.

Observations with excessive leverage but minimal influence are very unlikely to have an important effect on the substantive inferences of the analysis. In consequence, this analysis (based on the manually generated δβs) can be considered to have addressed the crucial elements of both influence and leverage.

The modelling process was carried out for all three exposures of primary interest and separate analyses were undertaken: (i) using all three matched controls per case and (ii) using only those controls from the initial selection process.

Analyses were then repeated using the additional results from the hair analysis. As there was no confirmatory assay available for cannabinoids using LC-MS/MS, data concerning the use of cannabis from the hair analysis was excluded from further analysis. Hair analysis results that were positive for the use of class A or B drugs were added to the database. In subjects where both the hair analysis results and interviewed data were negative or positive for class A or B drug use the data were unchanged. In those where the hair analysis recorded a positive result for class A or B drug use these subjects were recorded as users of class A or B drugs and the data were reanalysed.

6.11.3 Attributable risk calculations

The odds ratio estimates from the logistic regression analysis and information about the prevalence of each exposure of interest were used in the standard way

(Elwood, 1998) to estimate the attributable risk of each exposure on the prevalence of gastroschisis. Equivalent attributable risk estimates were calculated for other potentially modifiable risk factors in the final model. These estimates were then recalculated incorporating the additional information collected from the hair analysis.

6.12 Research Ethics Committee approval and support for the study

Following a very lengthy process spanning 18 months and attendance at the Trent Multi-Centre Research Ethics Committee (MREC) ethics committee approval was obtained from the Trent MREC and the 37 Local Research Ethics Committees. This approval was timed to commence in January 2001 following receipt of funding for the study. In addition permission and indemnity was sought from the Research and Development offices of all 69 NHS trusts involved in the study. All obstetricians, neonatologists and paediatric surgeons, in the three regions, were approached for their support at the start of the study.

6.13 Funding

This study was funded by a NHS Executive Trent Research and Development grant with contributions from the NHS Executive R&D West Midlands and NHS Exec R&D Northern Regions.

Results: Part One – Descriptive Analysis of the Gastroschisis Cases and Their Matched Controls.

7.1 Prevalence of Gastroschisis

During the period of the study, 1st January 2001 to 31st August 2003 there were 165 cases of gastroschisis delivered to 164 mothers within the three study regions. This represents an overall prevalence of 4.24 per 10,000 total births (95%CI, 3.62 to 4.94) as shown in Table 7.1.

The majority of the 165 gastroschisis cases were isolated anomalies, 154 (93.3%) cases. However, 14 (8.5%) of the isolated cases had conditions associated with their gastroschisis or with preterm delivery (Table 7.2). The 11 (6.7%) gastroschisis cases with multiple anomalies had a range of anomalies with no one specific body system being affected. There were two (1.2%) cases with chromosomal anomalies (a Turners mosaic 45X/46XX and a triploidy, both with other associated anomalies); three (1.8%) had limb defects (a case of shortened forearms with syndactyly and finger/toe anomalies; a case of arthrogryposis with kyphosis; and one case with talipes); two (1.2%) had renal defects (hydronephrosis persisting at delivery); one (0.6%) case had an atrial septal defect and a ventricular septal defect; one (0.6%) case had anencephalus; and two (1.2%) cases had multiple anomalies (one with asplenia, brachydactyly and low set ears and the other with a cleft lip and low set ears).

Table 7.1: Overall prevalence and birth prevalence of gastroschisis by year by region, January 2001 to August 2003.

Region	2001	2002	Jan-Aug 2003	Total Study Period
Trent				
Total births	54302	54601	37097	146000
Gastroschisis cases	24	27	16	67
Overall Prevalence	4.42	4.94	4.31	4.59
(95% ci)	*(2.83 - 6.58)*	*(3.26 – 7.19)*	*(2.47 – 7.00)*	*(3.56 – 5.83)*
Birth prevalence	3.87	4.76	3.77	4.18
(95% ci)	*(2.39 – 5.91)*	*(3.11 – 6.98)*	*(2.06 – 6.33)*	*(3.20 – 5.37)*
West Midlands				
Total births	61155	61418	42425	164998
Gastroschisis cases	22	25	18	65
Overall Prevalence	3.60	4.07	4.24	3.94
(95% ci)	*(2.25 – 5.45)*	*(2.63 – 6.01)*	*(2.51 – 6.70)*	*(3.04 – 5.02)*
Birth prevalence	3.27	3.91	4.24	3.76
(95% ci)	*(2.00 – 5.05)*	*(2.50 – 5.81)*	*(2.51 – 6.70)*	*(2.88 – 4.82)*
Northern				
Total births	29059	29394	19924	78377
Gastroschisis cases	11	7	15	33
Overall Prevalence	3.79	2.38	7.53	4.21
(95% ci)	*(1.89 – 6.77)*	*(0.96 – 4.91)*	*(4.21 – 12.41)*	*(2.90 – 5.91)*
Birth prevalence	3.79	2.38	7.53	4.21
(95% ci)	*(1.89 – 6.77)*	*(0.96 – 4.91)*	*(4.21 – 12.41)*	*(2.90 – 5.91)*
All Three Regions				
Total Births	144516	145413	99446	389375
Gastroschisis cases	57	59	49	165
Overall Prevalence rate	3.94	4.06	4.93	4.24
(95% ci)	*(2.99 – 5.11)*	*(3.09 – 5.23)*	*(3.65 – 6.51)*	*(3.62 – 4.94)*
Birth prevalence rate	3.60	3.92	4.73	4.01
(95% ci)	*(2.69 – 4.72)*	*(2.97 – 5.08)*	*(3.47 – 6.28)*	*(3.40 – 4.69)*

Table 7.2 Anomalies associated with gastroschisis

Gastroschisis case type	Associated conditions / anomalies	n (total=165)	%
Isolated	**None**	**140**	***84.8***
	patent ductus arteriosus associated with preterm delivery	4	*2.4*
	cryptorchidism	3	*1.8*
	antenatally diagnosed hydronephrosis resolved at delivery	2	*1.2*
	bowel problems associated with gastroschisis	5	*3.0*
	Total isolated with associated conditions	**14**	***8.5***
Multiple	Chromosomal	2	*1.2*
	Limb defects	3	*1.8*
	Renal	2	*1.2*
	Cardiac defect	1	*0.6*
	Neural tube defect	1	*0.6*
	Multiple	2	*1.2*
	Total associated with unrelated anomalies	**11**	***6.7***

7.2 Response

7.2.1 Response of the gastroschisis mothers

Of the 164 eligible mothers whose pregnancies resulted in at least one fetus or infant with gastroschisis, 144 mothers agreed to participate in the study; a response of 87.8% (Table 7.3).

Table 7.3: Response of gastroschisis mothers by region of residence.

Region	Eligible cases	Responders	%
Trent	67	61	*91.0*
West Midlands	64	53	*82.8*
Northern	33	30	*90.9*
Overall response	164	144	*87.8*

There were 20 cases of gastroschisis who were non-responders. Of these 15 (75.0%) refused, one (5.0%) was identified too late for inclusion and in the other four (20.0%) cases there were problems with accessing the patient either by their

non-attendance at appointments for antenatal and postnatal care or lack of co-operation of the health professionals involved in their care.

Table 7.4 shows that the maternal and pregnancy characteristics of the responding and non-responding gastroschisis mothers were not significantly different in terms of maternal age, ethnicity and multiplicity of pregnancy. However, although the median gestational age at delivery for the non-responding mothers was the same as for responding mothers there were significantly different ranges of gestational age in the two groups with a wider spread in the non-responding

Table 7.4: Maternal and pregnancy characteristics for gastroschisis mothers by response status.

Characteristic	Responders n=144 median	*IQR*	Non responders n=20 median	*IQR*	*p value*
Maternal age (years)	20.9	*19.1 – 24.7*	20.2	*18.5 – 24.7*	*0.419* [a]
Gestational age (wks)	36.0	*35.0 – 37.0*	36.0	*25.5 – 37.0*	***0.0012*** [a]
	n	*%*	n	*%*	
Singleton	141	*97.9*	20	*100.0*	*1.00* [b]
Ethnic group:					
White	131	*91.0*	17	*85.0*	
Asian	3	*2.1*	1	*5.0*	*0.418* [b]
All Black	2	*1.4*	0	-	
Mixed/other	8	*5.5*	0	-	
Unknown	0	-	2	*10.0*	

[a] *Mann Whitney U test*
[b] *Chi squared test – Fishers exact test where necessary for small numbers.*

mothers, especially at the lower gestational ages. This is explained in Table 7.5 where the characteristics of the gastroschisis fetuses and infants are described, indicating that significantly more of the pregnancies of non-responding mothers ended in termination; 25.0% in the non-responding mothers compared to 2.8% in the responding mothers. Indeed, a positive outcome, in terms of survival to one year of age, was experienced by the vast majority of infants of the responding mothers (93.0%) compared with only a little over half (55.0%) of the non-

responding mothers. An overall lower median birth weight and distribution of birth weights are also seen in the non-responding cases. Although this difference was not statistically significant it can also be explained by the difference in pregnancy outcome.

Table 7.5: Birth characteristics for gastroschisis cases by response status.

Characteristic	Responders n=145 median	*IQR*	Non responders n=20 median	*IQR*	*p value*
Birthweight (g)	2345	*2010 – 2665*	2070	*350 – 2238*	*0.07*[a]
	n	%	n	%	
					X^2
Outcome					
Termination	4	*2.8*	5	*25.0*	***<0.0001***[b]
Stillbirth	4	*2.8*	2	*10.0*	
Neonatal death	1	*0.7*	2	*10.0*	
Post neonatal death	1	*0.7*	0	-	
One year survivor	135	*93.0*	11	*55.0*	
Isolated anomaly	140	*96.6*	14	*70.0*	***<0.0001***[b]
Male	77	*53.1*	11	*55.0*	
Female	68	*46.9*	8	*40.0*	*0.81*[b]
Unknown	0	-	1	*5.0*	

[a] *Mann Whitney U test*
[b] *Chi squared test – Fishers exact test where necessary for small numbers.*

In both the responders and non-responders, the outcome for the multiple anomaly cases was divided between mid-trimester termination of pregnancy (2 responders and 3 non-responders) and those still alive at one year of age (3 responders and 3 non-responders). Two (1.4%) of the responding cases died: one as an early neonatal death on the second day and one as a post neonatal death half way through the fourth month. Both deaths in the non-responders occurred in the neonatal period, one in the first day and one on day ten. There were similar proportions of male and female infants in both response groups.

Out of the total 165 individual fetuses with gastroschisis (responders and non-responders) 158 (95.8%) had the condition diagnosed ante-natally; 140 (96.6%) were responders and 18 (90.0%) non-responders. Of the remaining five

responding cases two were suspected as having exomphalos; one case was described as having an unspecified abdominal wall defect; one case had an associated congenital hydronephrosis and the remaining case had no anomaly ante-natally detected. In the two remaining non-responding cases, whilst other anomalies were ante-natally detected the gastroschisis was not.

7.2.2 Response of the matched control mothers

Three controls, matched for maternal age, initial intended place of delivery and region of residence, were selected for each gastroschisis case. If a control mother refused to participate then further mothers were selected until the full complement of controls were recruited for each case. Table 7.6 shows the total number of control mothers required and the number approached to reach the required number by region. The response rate for control mothers who were originally approached was 76.9%, ranging from 71.0% in the West Midlands to 83.3% in the Northern region.

Table 7.6: Response of matched control mothers by region of residence.

Region	Number of controls required	Total controls approached to reach the required number	Original Response %
Trent	183	230	*79.6*
West Midlands	90	108	*83.3*
Northern	159	224	*71.0*
Overall response	432	562	*76.9*

Due to patient confidentiality and ethical issues additional data could not be collected about the non-responding controls. The only information known about this group of women was their age, initial intended or intended place of delivery and their region of residence as these variables were used for the matching process.

7.3 Matching variables for the main case-control study

All control mothers were successfully matched to gastroschisis case mothers for their region of residence. The median age was 21.3 years (IQR 19.4 to 25.2) for the control mothers and 20.9 years (IQR 19.1 to 24.7) for the gastroschisis case mothers indicating that matching on this variable was also successfully achieved (p=0.452).

Table 7.7: Matching variable initial intended place of delivery: directly matched gastroschisis cases and controls and level of matching where a direct match was unavailable.

Controls n=432		
n	**%**	**Level of matching**
401	*92.8*	Directly matched to initial intended place of delivery
15	*3.5*	Matched to intended place of delivery
4	*0.9*	Matched to place of delivery
12	*2.8*	Nearest hospital to initial intended place of delivery used as no controls subjects available in initial intended place of delivery

Successful direct matching of the initial intended place of delivery of the controls to the gastroschisis cases was achieved in 92.8% of all controls (Table 7.7). The initial intended place of delivery was defined as the hospital where the mother was initially booked for delivery. In three gastroschisis cases the initial intended place of delivery was outside the study regions. The nine controls selected for these cases were therefore selected from the intended place of delivery of the gastroschisis cases. The intended place of delivery was defined as the intended place of delivery of the mother at the onset of labour. Similarly in five controls their initial intended place of delivery was outside the study regions and therefore they were matched to the intended place of delivery of the gastroschisis cases. One control was initially booked for a home delivery but then transferred to an intended hospital delivery. This intended place of delivery was therefore matched to the initial intended delivery of the gastroschisis case. One case of gastroschisis was never booked for care and therefore the three controls for this case were matched

to the actual place of delivery of the case. Similarly one control mother who was never booked for delivery was matched by her actual place of delivery to the initial intended place of delivery of the gastroschisis case mother. Finally for 12 (2.8%) controls the nearest hospital to the initial intended place of delivery for the case was used because there were no regional residents of the correct age available for selection as controls in the required unit.

7.4 Characteristics of the gastroschisis cases and their matched controls

A description of the characteristics and putative risk factors for the cases and controls is presented in two sections. Section 7.4.1 is descriptive, providing information about the care received and outcome of pregnancy for the cases and controls. Section 7.4.2 provides information concerning any potential confounders and putative risk factors for inclusion in the matched case-control analysis. Univariate test statistics are not presented as the data come from matched sets.

7.4.1 Pregnancy care, labour, delivery and outcome factors

Care provision during pregnancy was similar for the gastroschisis cases and the controls in terms of the receipt of antenatal care, first attendance for antenatal care, the timing and provision of an anomaly scan and antenatal alpha feto-protein screening (Table 7.8). Prenatal diagnostic investigation was more common in gastroschisis cases with 7.6% of cases having had an amniocentesis compared to 1.2% of controls.

Table 7.8: Pregnancy care characteristics for gastroschisis case mothers and the controls.

Characteristics	Gastroschisis n=144		Controls n=432	
Attendance for ANC	143	*99.3%*	428	*99.1%*
Weeks gestation at 1st ANC (median / IQR)	10	*9 - 13*	10	*8 - 13*
Mid-trimester anomaly scan carried out	143	*99.3%*	420	*97.2%*
Gestation at U/S scan (median / IQR)	18	*17 - 20*	20	*19 - 20*
AFP test	66	*45.8%*	220	*50.9%*
Amniocentesis	11	*7.6%*	5	*1.2%*

Half of the gastroschisis cases were delivered following a spontaneous labour compared with just less than three-quarters of the control mothers (Table 7.9). Conversely, labour was induced for nearly twice as many gastroschisis cases (41.4% compared with 22.0% controls) or their mothers did not labour (9.6% compared with 4.4%). Over two-fifths (42.8%) of gastroschisis cases were delivered by caesarean section, the majority of which were unplanned. In the controls nearly three-quarters (71.3%) had a normal vaginal delivery and only 15.5% were delivered by caesarean section. Over a quarter (28.4%) of these caesarean sections were planned.

Fetal distress during labour and delivery was recorded in the notes of 37.5% of gastroschisis cases compared with only 11.3% of controls. This finding was reflected in some of the clinical features of fetal distress with 44.4% gastroschisis cases presenting with meconium stained liquor and 13.8% having type II dips recorded on intrapartum cardiotocographs compared to 10.9% and 7.4%, respectively, in the controls. Similar levels of intrapartum bradycardia and low scalp pH were found in both the gastroschisis cases and the controls. Overall, however, the apgar scores for the live born infants, at both one and five minutes post delivery, were very similar.

Table 7.9: Labour, delivery and outcome characteristics for gastroschisis case and control infants

Characteristics	Gastroschisis cases n=145		Controls n=432	
	n	%	n	%
Type of labour				
Spontaneous	71	*49.0*	318	*73.6*
Induced	60	*41.4*	95	*22.0*
Did not labour	14	*9.6*	19	*4.4*
Mode of delivery				
Normal	73	*50.3*	308	*71.3*
Instrumental	10	*6.9*	57	*13.2*
Elective CS	9	*6.2*	19	*4.4*
Emergency CS	53	*36.6*	48	*11.1*
Singleton	141	*97.2*	431	*99.8*
Twin	4	*2.8*	1	*0.2*
Male	77	*53.1*	210	*48.6*
Fetal Distress during labour & delivery				
Fetal distress	54	*37.2*	49	*11.3*
Meconium stained liquor	64	*44.1*	47	*10.9*
Type II dips	20	*13.8*	32	*7.4*
Bradycardia (HR<100)	13	*9.0*	45	*10.4*
Scalp pH <7.2	2	*1.4*	6	*1.4*

Excluding TOPs	**n=140**		**n=432**	
	median	*IQR*	median	*IQR*
Birth weight	2350	*2025 – 2665*	3360	*3010 – 3660*
Gestation	36	*35 – 37*	40	*39 – 40.5*
Apgar score at 1 minute	8	*7 – 9*	9	*8 – 9*
Apgar score at 5 minutes	9	*9 – 10*	9	*9 - 10*

The majority of both the gastroschisis cases and the controls were singletons. There was a small excess of male infants in the gastroschisis cases; this was not reflected in the controls (53.1% and 48.6%, respectively). As expected (after excluding cases delivered following termination of pregnancy) the median gestational age at delivery was lower in the gastroschisis cases than the controls; 36 weeks compared to 40 weeks. Consequently, the median birth weight was also lower: 2350 grammes and 3360 grammes, respectively.

7.4.2 Potential confounders and putative risk factors

The overall ethnic mix of the cases and controls was very similar (Table 7.10). In terms of parity, however, differences can be seen with slightly more primiparous women and those with two or more previous pregnancies within the gastroschisis case group and more women only one previous pregnancy in the control group.

Table 7.10: Maternal characteristics of the cases and controls

Characteristics	Gastroschisis cases n=144		Controls n=432	
	n	**%**	**n**	**%**
Ethnic group				
White	131	*91.0*	395	*91.4*
Asian	3	*2.1*	11	*2.6*
Black	2	*1.4*	16	*3.7*
Mixed / other	8	*5.5*	10	*2.3*
Parity				
Primiparous	99	*68.8*	277	*64.1*
1	24	*16.7*	108	*25.0*
2 or more	21	*14.5*	47	*10.9*
Marital Status				
Married	21	*14.6*	117	*27.1*
Single	121	*84.0*	308	*71.3*
Wid / Sep / Divorced	2	*1.4*	7	*1.6*
	median	***IQR***	**median**	***IQR***
Maternal Height (cms)	165	*160 - 170*	163	*158 - 168*
Pre-pregnancy weight (Kg)	57	*52 - 64*	61	*54 - 70*
Body Mass Index	21.1	*19.4 – 22.9*	22.5	*20.3 – 26.0*

Only a very small proportion of the gastroschisis case mothers were married (14.6%). This proportion was approximately half of that in the control women (27.1%). The median height of the cases and controls was very similar whilst the median pre-pregnancy weight of the gastroschisis case mothers was a little less than the control mothers. This was reflected in the median body mass index (BMI) of the two groups with a lower median BMI in the case mothers.

Table 7.11: Socio-economic characteristics of the gastroschisis case mothers and the controls

Characteristics	Gastroschisis cases n=144		Controls n=432	
	n	%	n	%
Unsupported	31	*21.5*	118	*27.3*
Mother worked during pregnancy	87	*60.4*	275	*63.6*
Maternal Social Class				
I, II, IIInm*	52	*36.1*	165	*38.2*
IIIm, IV, V	35	*24.3*	110	*25.4*
Housewife/Unemployed	57	*39.6*	157	*36.4*
Aggregate[!] Social Class				
I, II, IIInm*	56	*38.9*	195	*45.1*
IIIm, IV, V	54	*37.5*	168	*38.9*
Unemployed	34	*23.6*	69	*16.0*
Homeowner	36	*25.0*	170	*39.4*
Use of car	95	*66.0*	306	*70.8*

** includes students*
[!]Aggregate social class is the highest social class of the mother or partner in a supported relationship and the mother's social class in an unsupported relationship.

The proportion of financially unsupported mothers in the control group was greater than in the gastroschisis case mothers; 27.3% compared to 21.5% (Table 7.11). Other socio-economic factors did, however, follow a pattern of lower socio-economic status in the case mothers. Slightly more of the controls mothers worked during pregnancy, a fact reflected in the small differences in the maternal social class. In terms of aggregate social class (ie the highest social class of the mother or partner in a supported relationship and the mother's social class in an unsupported relationship) there were fewer higher social class (ie social class I, II & III non manual) and more unemployed cases than controls. Only a quarter of gastroschisis case mothers were homeowners compared to nearly two-fifths of controls (39.4%). In addition, a slightly lower proportion of gastroschisis case mothers had the use of a car (66.0% compared to 70.8%).

None of the women had a history of diabetes and very few women in either group had a history of epilepsy (Table 7.12). The prevalence of anaemia and

depression, although low, was higher in the gastroschisis case mothers than in the controls: 4.9% compared with 3.2% and 9.0% compared with 6.0%, respectively.

Table 7.12: General medical history, any medical complications during pregnancy and any family history of anomalies for gastroschisis case mothers and their controls

Medical History	Gastroschisis cases n=144		Controls n=432	
	n	%	n	%
General:				
Diabetes	0	-	0	-
Epilepsy	3	*2.1*	7	*1.6*
Anaemia	7	*4.9*	14	*3.2*
Asthma	18	*12.5*	66	*15.3*
Depression	13	*9.0*	26	*6.0*
Chlamydia	8	*5.6*	7	*1.6*
Recurrent urinary tract infections	5	*3.5*	9	*2.1*
Abnormal smear	6	*4.2*	6	*1.4*
Recreational drug use	12	*8.3*	4	*0.9*
Gynaecological disease/infection	18	*12.5*	22	*5.1*
During pregnancy:				
Anaemia	6	*4.2*	25	*5.8*
Antepartum haemorrhage	10	*6.9*	54	*12.5*
Infection	7	*4.9*	23	*5.3*
Premature rupture of membranes	10	*6.9*	8	*1.9*
Oligohydramnios	22	*15.3*	7	*1.6*
Intra-uterine growth restriction	10	*6.9*	7	*1.6*
Family History of Anomalies:				
Central nervous system	4	*2.8*	17	*3.9*
Cardiac	3	*2.1*	4	*0.9*
Eye & Ear	1	*0.7*	2	*0.5*
Alimentary	1	*0.7*	4	*0.9*
Musculo-skeletal	2	*1.4*	4	*0.9*
Chromosomal	4	*2.8*	5	*1.2*
Other	0	-	4	*0.9*
Any	15	*10.4*	36	*8.3*

Asthma prevalence was slightly lower in the gastroschisis case mothers; 12.5% compared with 15.3%. There were higher proportions of case mothers with a history of chlamydia, recurrent urinary tract infection and abnormal smears. Aggregating these three gynaecological conditions into a single variable showed that over twice as many cases as controls were affected (12.5% and 5.1%, respectively). A history of recreational drug use was recorded in the notes of a

much larger proportion of gastroschisis case mothers than in the controls: 8.3% compared with 0.9%.

There were higher recorded levels of anaemia, infection and, in particular, antepartum haemorrhage in the control mothers. In contrast, premature rupture of the membrane and intra-uterine growth restriction were approximately four times more common and oligohydramnios was ten times more common in the case mothers, 15.3% compared with 1.6% in control mothers.

A first degree family history of anomalies was rare in both the cases and the controls (10.4% and 8.3%, respectively). Central nervous system anomalies were more frequently found in the family history of control mothers (3.9% vs 2.8%), whereas a family history of chromosomal anomalies was more common in the gastroschisis case mothers (2.8% vs 1.2%).

Of the mothers who had had a previous pregnancy only one gastroschisis case mother had had a previous pregnancy complication by the presence of anomalies. The infant resulting from this pregnancy had multiple anomalies including a gastroschisis and limb defects. Three control mothers had had a pregnancy affected by anomalies: stenosis of the pulmonary artery, cystic fibrosis and a major partial trisomy.

Eighty-seven (60.4%) case mothers and 275 (63.7%) control mothers worked during pregnancy. There were no striking differences in the proportions of gastroschisis case mothers and the control mothers in terms of their occupational or industrial groupings (Table 7.13). A slightly higher proportion of control mothers worked in conditions that they reported as either very hot or noisy when compared to the case mothers. Although the proportion of case mothers who reported working in a very dirty environment was higher than in the control mothers, numbers are very small; only 3 case mothers and 4 control mothers.

Table 7.13: Occupation, industry and working conditions for the gastroschisis case mothers and the control mothers who worked during pregnancy

Characteristics	Gastroschisis cases n=87		Controls n=275	
	n	%	n	%
Occupational group:				
Manager	8	*9.2*	25	*9.1*
Health / welfare	13	*14.9*	48	*17.5*
Clerical	14	*16.1*	47	*17.1*
Catering	10	*11.5*	20	*7.3*
Hair / beauty	5	*5.7*	13	*4.7*
Sales	20	*23.0*	45	*16.4*
Factory	2	*2.3*	13	*4.7*
Other	15	*17.2*	64	*23.3*
Industry:				
Engineering	6	*6.9*	17	*6.2*
Food processing	3	*3.4*	5	*1.8*
Distribution	33	*37.9*	84	*30.5*
Transport / communication	8	*9.2*	24	*8.7*
Bank / finance	10	*11.5*	27	*9.8*
Other	27	*31.0*	118	*42.9*
Working conditions:				
Very noisy	7	*8.0*	34	*12.4*
Very hot	5	*5.7*	22	*8.0*
Very dirty	3	*3.4*	4	*1.5*

Overall similar proportions of case and control mothers were exposed to a variety of chemicals during their pregnancies, either in the workplace or as part of a hobby (Table 7.14). The proportion of mothers exposed to solvents was virtually the same in both groups with slightly higher proportions of controls mothers being exposed to glues or adhesives, cleaning agents and colour mixing solutions and slightly higher proportions of gastroschisis mothers being exposed to spray paints and other chemicals. Photocopier and visual display unit (VDU) use was higher in the controls mothers. Nearly 90% of all mothers in both groups used a microwave oven during pregnancy and less than 4% used an ultrasound device.

A higher proportion of gastroschisis case mothers were exposed to X-rays in the first trimester of pregnancy (2.1% compared with 0.7% control mothers). These were for purposes unrelated to their pregnancy, primarily dental X-rays, in a very small number of women.

Table 7.14: Occupational and hobby chemical and equipment exposures during pregnancy for gastroschisis case mothers and their controls

Exposures	Gastroschisis cases n=144		Controls n=432	
	n	%	n	%
Exposures (work and/or hobby):				
Solvents	21	*14.6*	62	*14.4*
Glues/adhesives	8	*5.6*	56	*13.0*
Cleaning agents	70	*48.6*	222	*51.4*
Paint spraying	9	*6.3*	21	*4.9*
Colour mixing solutions	4	*2.8*	19	*4.4*
Other chemicals	19	*13.2*	45	*10.4*
Use of equipment (work and/or home):				
Photocopier				
VDU	28	*19.4*	117	*27.1*
Microwave	53	*36.8*	208	*48.2*
Ultrasound device	129	*89.6*	376	*87.0*
	5	*3.5*	16	*3.7*
Exposure to X-rays during the first trimester	3	*2.1*	3	*0.7*

Table 7.15: The use of prescribed and 'over the counter' medicines in the first trimester for gastroschisis case mothers and their controls

Medications	Gastroschisis cases n=144		Controls n=432	
	n	%	n	%
Aspirin	7	*4.9*	2	*0.5*
Paracetamol	65	*45.1*	191	*44.2*
Ibuprofen	9	*6.3*	20	*4.6*
Antihistamine	1	*0.7*	2	*0.5*
Ephedrine	1	*0.7*	0	-
Phenlypropanolamine	3	*2.1*	4	*0.9*
Oral contraceptive	9	*6.3*	15	*3.5*
Antidepressants	6	*4.2*	6	*1.4*

The results relating to the use of prescribed and 'over the counter' (OTC) medication in early pregnancy are presented in Table 7.15. Although only a few mothers used aspirin during early pregnancy there was a ten-fold difference in the proportions between the case and control mothers: 4.9% compared with 0.5%. Similar proportions of the mothers in either group used paracetamol in early pregnancy. Use of antihistamine and ephedrine was very rare. Higher proportions of the case mothers used ibuprofen, phenylpropanolamine, oral contraceptives

and antidepressants in early pregnancy then the control mothers. These proportions were, however, small; the greatest being 6.3% of gastroschisis case mothers using ibuprofen and oral contraceptives in early pregnancy.

First trimester consumption behaviours show that caffeine consumption (all sources) was similar in the case and control mothers except in those drinking twenty or more cups per day where 12.5% of case mothers reported this level of consumption compared with 8.1% of control mothers (Table 7.16). Over two-thirds (69.4%) of all gastroschisis case mothers reported smoking in the first trimester, of whom one-third smoked more than ten cigarettes per day. Substantially less control mothers (49.8%) reported smoking in early pregnancy, but of those who did smoke, as in the cases, one-third smoked more than ten cigarettes per day. Alcohol intake was slightly higher in the gastroschisis case mothers compared to their matched controls with 27.1% of case mothers reporting drinking 14 or more units per week compared to 19.4% of control mothers. Binge drinking was, however, reported by similar proportions of women in each group with 8.4% of case mothers reporting drinking 11 or more units on one occasion during early pregnancy compared with 7.9% of control mothers.

Reported recreational drug use during the first trimester of pregnancy was higher, in all categories, for the case mothers. The prevalence of any recreational drug use in early pregnancy was three times higher in the case mothers overall compared with the controls: 16.7% compared with 5.5%, respectively. Two or more recreational drugs were used by 8.4% of case mothers compared with 0.4% of control mothers and nearly one-tenth of all gastroschisis case mothers (9.7%) reported the use of a Class A or B drug during early pregnancy compared with only 1.9% of control mothers.

Table 7.16: Self-reported consumption behaviours during the first trimester for gastroschisis case mothers and their controls

Factors		Gastroschisis n=144		Controls n=432	
		n	%	n	%
Caffeine consumption:					
	None	15	*10.4*	43	*10.0*
	1 to 4 cups	38	*26.4*	124	*28.7*
	5 to 9 cups	37	*25.7*	119	*27.5*
	10 to 19 cups	36	*25.0*	111	*25.7*
	20 or more cups	18	*12.5*	35	*8.1*
Smoker		100	*69.4*	215	*49.8*
Cigarettes per day:					
	None	44	*30.6*	217	*50.2*
	1 to 10	67	*46.5*	146	*33.8*
	More than 10	33	*22.9*	69	*16.0*
Consumed Alcohol		99	*68.8*	259	*60.0*
Weekly alcohol consumption:					
	None	45	*31.2*	173	*40.0*
	Less than 14 units	60	*41.7*	175	*40.6*
	14 to <28 units	22	*15.3*	48	*11.1*
	28 or more units	17	*11.8*	36	*8.3*
Binge drinking:	**None**	126	*87.4*	384	*88.9*
	6 to 10 units	6	*4.2*	14	*3.2*
	11 to 20 units	9	*6.3*	25	*5.8*
	more than 20 units	3	*2.1*	9	*2.1*
Binge drinker		18	*12.6*	48	*11.1*
Recreational drug use[$]:	**None**	120	*83.3*	409	*94.7*
	Heroin	6	*4.2*	1	*0.2*
	Cocaine	2	*1.4*	1	*0.2*
	Ecstasy	7	*4.9*	4	*0.9*
	Amphetamine	5	*3.5*	3	*0.7*
	Cannabis	21	*14.6*	18	*4.2*
Any recreational drug use:		24	*16.7*	23	*5.3*
	One drug only	12	*8.3*	21	*4.9*
	Two drugs	9	*6.3*	1	*0.2*
	Three or more drugs	3	*2.1*	1	*0.2*
Class A or B drug		14	*9.7*	8	*1.9*

[$] *more than one type of recreational drug may be recorded*

Table 7.17: Putative risk factors by recreational drug status for gastroschisis mothers and their controls.

Factor	Gastroschisis Recreational drugs -ve n	%	Gastroschisis Recreational drugs +ve n	%	Controls Recreational drugs -ve n	%	Controls Recreational drugs +ve n	%
First trimester alcohol use:								
None	39	*32.5*	6	*25.0*	169	*41.3*	4	*17.4*
<14 units / week	51	*42.5*	9	*37.5*	164	*40.1*	11	*47.8*
14 +units / week	30	*26.3*	9	*37.5*	76	*18.6*	8	*34.7*
First trimester cigarette use:								
None	41	*34.2*	6	*25.0*	212	*51.8*	6	*26.1*
1-10 cigs / day	53	*44.2*	11	*45.8*	135	*33.0*	10	*43.5*
10+ / day	26	*21.7*	7	*29.2*	62	*15.2*	7	*30.4*
First trimester aspirin use:								
No	114	*95.0*	23	*95.8*	407	*99.5*	23	*100.0*
Yes	6	*5.0*	1	*4.2*	2	*0.5*	0	*0.0*
Body mass index:								
Normal	87	*72.5*	16	*66.7*	242	*59.2*	18	*78.3*
Underweight	20	*16.7*	6	*25.0*	39	*9.5*	2	*8.7*
Overweight / obese	13	*10.9*	2	*8.3*	128	*31.3*	3	*13.0*
Marital status:								
Yes	19	*15.8*	2	*8.3*	116	*28.4*	1	*4.3*
No	101	*84.2*	22	*91.7*	293	*71.6*	22	*95.7*
Homeowner:								
Yes	34	*28.3*	3	*12.5*	164	*40.1*	6	*26.1*
No	86	*71.7*	21	*87.5*	245	*59.9*	17	*73.9*
Gynaecological infection / disease:								
No	107	*89.2*	19	*79.2*	387	*94.6*	23	*100.0*
Yes	13	*10.8*	5	*20.8*	22	*5.4*	0	*0.0*
Total	**120**	***100.0***	**24**	***100.0***	**409**	***100.0***	**23**	***100.0***

Tables 7.17 and 7.18 show the relationship between the main putative risk factors for the study by the main exposures of interest: first trimester use of any recreational drug and first trimester use of a class A or B drug. Recreational drug users are more likely to consume both any and larger quantities of alcohol and cigarettes in the first trimester of pregnancy in both cases and controls, eg. only 26.3% of cases and 18.6% of controls who did not take recreational drugs consumed 14 or more units of alcohol per week compared to 37.5% of cases and 34.7% of controls who did take recreational

drugs. This relationship was stronger in the class A or B drug users with 57.1% of cases and 50.0% of controls reporting the consumption of 14 or more units of alcohol per week. There was only one first trimester aspirin user who also consumed any recreational drugs (none in the class A or B drug users) and this was a gastroschisis mother, the remaining six cases and two controls who took aspirin did not report taking any recreational drugs. Although there appears to be the suggestion of a relationship between recreational drug use and being underweight in the gastroschisis cases (25.0% vs 16.7%) this relationship is not present in the controls (8.7% vs 9.5%). The majority of recreational drug users (91.7% cases and 95.7% controls) and all class A or B drug users were unmarried. Recreational drug users were less likely to be a home-owner than those who did not take drugs. This relationship was stronger in the class A or B drug users, particularly the gastroschisis mothers (92.9% and 62.5% in gastroschisis mothers and controls, respectively) but was based on small numbers. There was no clear relationship between recreational drug use and a history of gynaecological infection or disease.

Table 7.18: Putative risk factors by class A or B drug status for gastroschisis mothers and their controls.

Factor	Gastroschisis Class A or B drugs -ve n	%	+ve n	%	Controls Class A or B drugs -ve n	%	+ve n	%
First trimester alcohol use:								
None	42	*32.3*	3	*21.4*	172	*40.6*	1	*12.5*
<14 units / week	57	*43.8*	3	*21.4*	172	*40.6*	3	*37.5*
14 +units / week	31	*23.8*	8	*57.1*	80	*18.9*	4	*50.0*
First trimester cigarette use:								
None	45	*34.6*	2	*14.3*	216	*50.9*	2	*25.0*
1-10 cigs / day	58	*44.6*	6	*42.9*	143	*33.7*	2	*25.0*
10+ / day	27	*20.8*	6	*42.9*	65	*15.3*	4	*50.0*
First trimester aspirin use:								
No	123	*94.6*	14	*100.0*	422	*99.5*	8	*100.0*
Yes	7	*5.4*	0	*0.0*	2	*0.5*	0	*0.0*
Body mass index:								
Normal	95	*73.1*	8	*57.1*	254	*59.9*	6	*75.0*
Underweight	20	*15.4*	6	*42.9*	40	*9.4*	1	*12.5*
Overweight / obese	15	*11.5*	0	*0.0*	130	*30.7*	1	*12.5*
Marital status:								
Yes	21	*16.2*	0	*0.0*	117	*27.6*	0	*0.0*
No	109	*83.8*	14	*100.0*	307	*72.4*	8	*100.0*
Homeowner:								
Yes	36	*27.7*	1	*7.1*	167	*39.4*	3	*37.5*
No	94	*72.3*	13	*92.9*	257	*60.6*	5	*62.5*
Gynaecological infection / disease:								
No	113	*86.9*	13	*92.9*	402	*94.8*	8	*100.0*
Yes	17	*13.1*	1	*7.1*	22	*5.2*	0	*0.0*
Total	**130**	***100.0***	**14**	***100.0***	**424**	***100.0***	**8**	***100.0***

Results: Part Two – Conditional Logistic Regression Analysis of the Gastroschisis Cases and Their Matched Controls

This chapter describes the results of the main case control analysis to test the primary hypothesis that recreational drug use in early pregnancy is associated with an increased risk of gastroschisis. Section 8.1 presents the results of the analysis in which all available controls per case were included. Section 8.2 presents the results of the analysis using only those controls that were initially selected for the study and consented to participate, i.e. the replacement controls were excluded from this analysis.

8.1 Matched case control analysis using the full complement of three controls per case.

This analysis is based upon the 144 gastroschisis mothers and their 432 matched controls (1:3 matching with replacement).

8.1.1 Conditional logistic regression analysis for individual risk factors – unadjusted odds ratios.

Mothers who were single, separated or divorced were over two and a half times more likely to have a gastroschisis pregnancy than their married control counterparts: (unadjusted odds ratio 2.59; 95% CI, 1.48 to 5.53), (Table 8.1). Mothers who were not homeowners (compared to homeowners) were also over two times more likely to have a gastroschisis pregnancy (unadjusted OR 2.38; 95% CI, 1.43 to 3.95). Similarly, those who were unemployed were nearly twice as likely to have a gastroschisis pregnancy than those mothers who were employed (unadjusted OR 1.78; 95% CI, 1.05 to 3.03).

Being overweight or obese was significantly protective against a gastroschisis pregnancy in the univariate analysis with the odds reduced to one-quarter that of a normal weight mother (unadjusted OR 0.23; 95% CI 0.09 to 0.59). In contrast, mothers who were underweight showed an increased odds of a gastroschisis pregnancy (unadjusted OR 1.70; 95% CI 0.97 to 2.99) although this narrowly failed to achieve statistical significance.

Table 8.1: Socio-demographic characteristics. Unadjusted odds ratios and 95% confidence intervals comparing cases to controls.

Characteristics		Unadjusted odds ratio	95% confidence interval
Ethnic group:	White	1.00*	
	Asian	0.34	0.07 to 1.56
	All Black	0.78	0.19 to 3.19
	Mixed / Other	1.67	0.40 to 7.03
Marital Status:	Married	1.00*	
	Single / separated / divorced	2.59	1.48 to 5.53[a]
Financial support status:	Supported	1.00*	
	Unsupported	0.68	0.41 to 1.12
Overall social class:	I, II or IIInM	1.00*	
	III, IV or V	1.13	0.74 to 1.74
	Unemployed	1.78	1.05 to 3.03[a]
Mother worked during pregnancy:	No	1.00*	
	Yes	1.28	0.86 to 1.91
Homeowner:	Yes	1.00*	
	No	2.38	1.43 to 3.95[a]
Car available for personal use:	Yes	1.00*	
	No	1.27	0.84 to 1.92
Body Mass Index:	Normal	1.00*	
	Underweight	1.70	0.97 to 2.99
	Overweight	0.32	0.16 to 0.64[b]
	Obese	0.23	0.09 to 0.59[b]

[a]Significantly elevated odds in gastroschisis mothers
[b]*Significantly reduced odds in gastroschisis mothers*

**Baseline comparison group*

There were no significant differences between cases and controls in terms of mothers ethnic group, financial support status, work during pregnancy or car availability.

The unadjusted odds ratio for taking aspirin during the first trimester of pregnancy indicated that there was a nineteen-fold excess risk of a gastroschisis pregnancy in these mothers (unadjusted OR 19.10) although the width of the 95% confidence interval for this exposure (2.33 to 156.45) reflected the fact that very few mothers actually took aspirin at this time (Table 8.2). Although the unadjusted odds ratios indicated that there was an increased risk of a gastroschisis pregnancy of between two and three fold in mothers who took phenylpropanolamine, anti-depressive drugs or oral contraceptives or were exposed to X-rays in the first trimester, these findings were, once again, based on small numbers and not statistically significant. Use of ibuprofen, antihistamines and paracetamol were not found to be significantly associated with gastroschisis pregnancies although their associated odds ratios were modestly elevated.

Table 8.2: First trimester exposure to prescribed or 'OTC' drugs, X-rays and medical history risk factors. Unadjusted odds ratio for gastroschisis mothers with 95% confidence intervals

Exposure present Yes:No		Unadjusted odds ratio	95% confidence interval
First trimester exposure to:			
	aspirin	19.10	2.33 to 156.45[a]
	ibuprofen	1.37	0.61 to 3.08
	antihistamines	1.50	0.14 to 16.54
	phenylpropanolamine	2.52	0.49 to 12.89
	paracetamol	1.04	0.70 to 1.54
	oral contraceptives	1.92	0.80 to 4.63
	anti-depressive drugs	3.00	0.97 to 9.30
	X-rays	2.25	0.50 to 10.05
History of depressive illness		1.67	0.78 to 3.54
History of gynaecological infection/disease[$]		2.78	1.41 to 5.46[a]
Family history of congenital anomalies		1.31	0.67 to 2.55
Parity:	Primiparous	1.67	0.97 to 2.88
	Parity 1	1.00*	
	Parity 2 or more	1.93	0.99 to 3.76

[a] *Significantly elevated odds in gastroschisis mothers*
[$] *History of chlamydia, recurrent urinary tract infections or abnormal cervical cytology*
** Baseline comparison group*

Medical history features are also presented in Table 8.2. Mothers who had a history of gynaecological infection or disease had nearly three times the risk of a gastroschisis pregnancy compared to those without such history (unadjusted OR 2.78; 95% CI, 1.41 to 5.46). Although a modestly increased odds of gastroschisis

was associated with a history of depressive illness and a family history of congenital anomalies neither were statistically significant. Similarly, the unadjusted odds ratio for mothers who had previously had either no previous pregnancies or two or more pregnancies compared to those who had only had one previous pregnancy indicated that such mothers over one and a half times the odds of a gastroschisis pregnancy, though this was not statistically significant.

Table 8.3: Consumption behaviours during the first trimester. Unadjusted odds ratios for gastroschisis mothers with 95% confidence intervals

Consumption behaviour Yes:No		Unadjusted odds ratio	95% confidence interval
First trimester caffeine use:	None	1.00*	
	1 to 9 cups / day	0.89	0.47 to 1.69
	10 to 19 cups / day	0.95	0.47 to 1.92
	20 or more cups / day	1.49	0.65 to 3.40
First trimester cigarette use:	None	1.00*	
	1 – 10 cigarettes per day	2.07	1.34 to 3.20[a]
	More than 10 cigarettes per day	2.27	1.34 to 3.85[a]
First trimester weekly alcohol consumption:	None	1.00*	
	Less than 14 units	1.33	0.85 to 2.09
	14 to <28 units	1.84	0.99 to 3.42
	28 or more units	1.88	0.96 to 3.66
First trimester binge drinking:	None	1.00*	
	6 to 10 units	1.30	0.50 to 3.38
	11 to 20 units	1.10	0.50 to 2.43
	more than 20 units	1.02	0.28 to 3.79
First trimester use of any recreational drug		3.37	1.85 to 6.13[a]
First trimester use of heroin		18.00	2.17 to 149.49[a]
First trimester use of cocaine		6.00	0.54 to 66.17
First trimester use of amphetamine		4.00	0.90 to 17.87
First trimester use of ecstasy		6.42	1.64 to 25.09[a]
First trimester use of cannabis		3.99	2.05 to 7.76[a]
First trimester use of a class A or B drug		5.76	2.32 to 14.31[a]

[a] *Significantly elevated odds in gastroschisis mothers*
**Baseline comparison group*

Table 8.3 presents the unadjusted odds ratios for the first trimester exposure of mothers for a variety of consumption behaviour patterns. Mothers who smoked during the first trimester of pregnancy had over twice the odds of a gastroschisis pregnancy compared to non-smoking mothers. There was a suggestion of a dose-response relationship with the number of cigarettes smoked, with the odds of

gastroschisis pregnancy being 2.07 (95% CI, 1.34 to 3.20) for those smoking ten or less cigarettes per day and the odds increasing to 2.27 (95% CI, 1.34 to 3.85) for those who smoked more than ten cigarettes per day. Although a dose response relationship was also found for first trimester caffeine and alcohol consumption neither of these showed a significantly raised odds for a gastroschisis pregnancy compared with controls. However, mothers who drank 14 or more units of alcohol per week during their first trimester showed an almost two-fold increased odds of a gastroschisis pregnancy although this was not statistically significant. Binge drinking during the first trimester did not increase a mother's odds of having a gastroschisis pregnancy.

Reported use of any recreational drug, either individually or in combination with other recreational drugs, was associated with over a three-fold odds of a gastroschisis pregnancy when compared with mothers who did not use recreational drugs. Although the results indicated that mothers who reported taking cocaine or amphetamines in the first trimester had a six-fold and four-fold excess odds, respectively, this was based on very small numbers and these results were not significant (cocaine unadjusted OR 6.00; 95% CI, 0.54 to 66.17 and amphetamine unadjusted OR 4.00; 95% CI, 0.90 to 17.87). Mothers who reported using cannabis, ecstasy or heroin in early pregnancy had a significantly increased odds of a gastroschisis pregnancy with unadjusted odds ratios of 3.99 (95% CI, 2.05 to 7.76), 6.42 (95% CI, 1.64 to 25.09) and 18.00 (95% CI, 2.17 to 149.49) respectively. There was, in excess of, a three-fold odds of having a gastroschisis pregnancy associated with the reported use of any recreational drug use during the first trimester of pregnancy (unadjusted OR 3.37; 95% CI, 1.85 to 6.13). This odds ratio increased to almost six-fold when considering the use of class A or B drugs alone (unadjusted OR 5.76; 95% CI, 2.32 to 14.31).

8.1.2 Conditional logistic regression adjusting for significant putative risk factors

A number of conditional logistic regression models were developed to determine the size of the risk of a gastroschisis pregnancy associated with the reported use of recreational drugs in the first trimester of pregnancy, having adjusted for potential confounding variables. All known or suspected confounders and putative

risk factors, presented in Tables 8.1 to 8.3, were entered into the conditional logistic regression model and removed in a stepwise manner to determine their effect on the fit of the data. Factors that did not have a significant effect on the fit of the data were removed. Factors that did significantly affect the fit of the model

Table 8.4: Mutually adjusted conditional logistic regression model for the reported use of any recreational drug during the first trimester of pregnancy.

Risk Factor		Adjusted[1] odds ratio	95% confidence interval
First trimester use of any recreational drug:	No	1.00*	
	Yes	2.22	1.15 to 4.29[a]
Body Mass Index:	Normal	1.00*	
	Underweight	2.10	1.15 to 3.85[a]
	Overweight	0.32	0.15 to 0.69[b]
	Obese	0.32	0.12 to 0.85[b]
Marital Status:	Married	1.00*	
	Single / separated / divorced	1.86	1.00 to 3.46[a]
First trimester use of aspirin:	No	1.00*	
	Yes	21.39	2.24 to 204.70[a]
Home owner:	Yes	1.00*	
	No	1.90	1.09 to 3.30[a]
History of gynaecological infection / disease:	No	1.00*	
	Yes	2.40	1.13 to 5.07[a]
First trimester cigarette use:	None	1.00*	
	1 – 10 cigarettes per day	1.63	1.00 to 2.63[a]
	More than 10 cigarettes per day	1.85	1.03 to 3.32[a]

[1] *Adjusted for all other characteristics in the table*
* *Baseline comparison group*
[a] *Significantly elevated risk in gastroschisis mothers*
[b] *Significantly reduced risk in gastroschisis mothers*

were returned to the model. Table 8.4 presents the final conditional logistic regression model for the reported use of any recreational drug during the first trimester of pregnancy in the gastroschisis mothers. Having taken into account confounding factors, mothers who reported using recreational drugs in the first trimester of pregnancy were over twice as likely to have a baby with a gastroschisis than mothers who did not report using recreational drugs at this stage of pregnancy (adjusted OR 2.22; 95% CI, 1.15 to 4.29).

The robustness of the model was then checked. Model checking procedures included: testing for interactions and none were found; removal of outliers, which had little effect upon the final estimate of the odds ratio (adjusted OR varied between 2.05 and 2.25); and testing for the relevant components of influence and leverage which, once again, had little effect on the estimated odds ratio (change from 2.22 to 2.19) and therefore the final model was considered to be robust.

The odds ratio for gastroschisis associated with the reported use of class A or B drugs during the first trimester of pregnancy was estimated (Table 8.5). After adjusting for maternal body mass index, marital status, aspirin use in the first trimester of pregnancy, home ownership, history of gynaecological infection or disease and smoking during the first trimester, there was a significant threefold excess risk of gastroschisis associated with the use of any class A or B drug (adjusted OR 3.59; 95% CI, 1.36 to 9.47).

Once again model checking revealed no significant interactions and the removal of outliers had minimal impact on the final estimate of the odds ratio. However, removal of observations with extreme $\delta\beta$s led to a near doubling of the adjusted odds ratio associated with the use of class A or B drugs in the first trimester of pregnancy. The estimated adjusted OR increased from 3.59 to 6.41. Such a phenomenon is not unusual in data sets of this size. Reassuringly, it is clear that the observations with excessive influence were shrinking rather than magnifying the odds ratio of interest, suggesting that one can be confident that the positive odds ratio upon which the primary inferences are based is not solely a consequence of unrepresentative observations with excessive influence.

Table 8.5: Mutually adjusted conditional logistic regression model for the reported use of a class A or B drug during the first trimester of pregnancy.

Risk Factor		Adjusted[1] odds ratio	95% confidence interval
First trimester use of a class A or B drug:	No	1.00*	
	Yes	3.59	1.36 to 9.47[a]
Body Mass Index:	Normal	1.00*	
	Underweight	1.99	1.08 to 3.70[a]
	Overweight	0.33	0.16 to 0.71[b]
	Obese	0.30	0.11 to 0.80[b]
Marital Status:	Married	1.00*	
	Single / separated / divorced	1.79	0.96 to 3.33
First trimester use of aspirin:	No	1.00*	
	Yes	20.50	2.18 to 192.29[a]
Home owner:	Yes	1.00*	
	No	2.02	1.16 to 3.52[a]
History of gynaecological infection / disease:	No	1.00*	
	Yes	2.64	1.25 to 5.59[a]
First trimester cigarette use:	None	1.00*	
	1 – 10 cigarettes per day	1.62	1.00 to 2.62[a]
	More than 10 cigarettes per day	1.75	0.96 to 3.18

[1] *Adjusted for all other characteristics in the table*
* *Baseline comparison group*
[a] *Significantly elevated risk in gastroschisis mothers*
[b] *Significantly reduced risk in gastroschisis mothers*

The risk associated with the use of recreational drugs during the first trimester of pregnancy in gastroschisis the mothers was estimated as a four level factor (Table 8.6). The four levels of recreational drug use were: (1) no use; (2) use of cannabis only; (3) use of class A or B drugs only; and (4) use of cannabis and a class A or B drug. Once again, after adjusting for maternal body mass index, marital status, first trimester aspirin use, home ownership, a history of gynaecological infection or disease and smoking there was a significant tenfold excess risk of gastroschisis associated with the use of cannabis and class A or B drugs during the first trimester of pregnancy (adjusted OR 10.86; 95% CI, 2.29 to 51.46). However, following adjustment for the other factors cannabis use alone and the use of class A or B drugs alone were not significantly associated with an excess risk of

gastroschisis (adjusted OR cannabis 1.40; 95% CI, 0.54 to 3.63; adjusted OR class A or B drugs 0.89; 95% CI, 0.19 to 4.22).

Table 8.6: Mutually adjusted conditional logistic regression model for the reported use of cannabis alone, class A or B drugs alone or both cannabis and class A or B drugs during the first trimester of pregnancy.

Risk Factor		Adjusted[1] odds ratio	95% confidence interval
First trimester use of an recreational drug:	No	1.00*	
	Cannabis	1.40	0.54 to 3.63
	Class A or B drugs	0.89	0.19 to 4.22
	Cannabis and class A or B drugs	10.86	2.29 to 51.46[a]
Body Mass Index:	Normal	1.00*	
	Underweight	2.09	1.11 to 3.92[a]
	Overweight	0.33	0.16 to 0.70[b]
	Obese	0.32	0.12 to 0.84[b]
Marital Status:	Married	1.00*	
	Single / separated / divorced	1.83	0.99 to 3.40
First trimester use of aspirin:	No	1.00*	
	Yes	20.76	2.19 to 196.44[a]
Home owner:	Yes	1.00*	
	No	1.87	1.07 to 3.26[a]
History of gynaecological infection / disease:	No	1.00*	
	Yes	2.71	1.26 to 5.80[a]
First trimester cigarette use:	None	1.00*	
	1 – 10 cigarettes per day	1.64	1.01 to 2.66[a]
	More than 10 cigarettes per day	1.66	0.91 to 3.05

[1] *Adjusted for all other characteristics in the table*
* *Baseline comparison group*
[a] *Significantly elevated risk in gastroschisis mothers*
[b] *Significantly reduced risk in gastroschisis mothers*

No significant interactions were found during the model checking process and the removal of outliers had little effect upon the final estimate of the odds ratio, for example (adjusted OR for the use of both cannabis and class A or B drugs in the first trimester between 9.29 and 11.06). Testing for the relevant components of influence and leverage indicated that there was little effect on the adjusted odds ratios for reported cannabis use alone or class A or B drug use alone in the first trimester (change in adjusted OR from 1.40 to 1.46 and 0.89 to 0.97, respectively). Unfortunately, it was not possible to estimate the adjusted odds ratio associated

with the use of both cannabis and class A or B drugs in the first trimester as no controls were exposed to both simultaneously. The estimated odds ratio for the joint effect was therefore infinite. This finding warrants further investigation in a larger study.

8.2 Analysis of Matched Case Control study using initially selected controls only – variable number of controls per case of between one and three.

The same basic analyses were carried out as before including only those control subjects that were initially selected for the study i.e. the first three controls selected for each case. Six case-control sets had to be discarded completely because none of the initially selected controls were recruited. A further 93 controls were excluded from this analysis as they were not within the initially selected sample. The analysis in this section is therefore based on 138 gastroschisis mothers and the 339 initially selected controls. Results using only those controls subjects initially selected are compared with those from the analysis using replacement controls.

8.2.1 Conditional logistic regression analysis for individual risk factors – unadjusted odds ratios.

A significantly raised odds of a gastroschisis pregnancy was found for the same socio-demographic characteristics using the initially selected controls as for the replacement control analysis (Table 8.7 compared with Table 8.1). The magnitude of the unadjusted odds ratios was very similar for all socio-demographic characteristics except for single, separated or divorced mothers whose odds of a gastroschisis pregnancy was increased from 2.59 to 3.56 (unadjusted ORs).

Table 8.7: Socio-demographic characteristics. Unadjusted odds ratios for gastroschisis – initially selected controls compared with all controls.

Characteristics		Unadjusted odds ratios	
		Initially selected controls	All controls
Ethnic group:	White	1.00*	1.00*
	Asian	0.38	0.34
	All Black	0.66	0.78
	Mixed / Other	1.38	1.67
Marital Status:	Married	1.00*	1.00*
	Single / separated / divorced	3.56[a]	2.59[a]
Financial support status:	Supported	1.00*	1.00*
	Unsupported	0.75	0.68
Overall social class:	I, II or IIInM	1.00*	1.00*
	III, IV or V	1.18	1.13
	Unemployed	1.78[a]	1.78[a]
Mother worked during pregnancy:	No	1.00*	1.00*
	Yes	1.28	1.28
Home owner:	Yes	1.00*	1.00*
	No	2.09[a]	2.38[a]
Car available for personal use:	Yes	1.00*	1.00*
	No	1.16	1.27
Body Mass Index:	Normal	1.00*	1.00*
	Underweight	1.44	1.70
	Overweight	0.31[b]	0.32[b]
	Obese	0.24[b]	0.23[b]

[a] *Significantly elevated odds in gastroschisis mothers*
[b] *Significantly reduced odds in gastroschisis mothers*
**Baseline comparison group*

Comparing the unadjusted odds ratios for first trimester exposure to prescribed or 'over the counter' medications and X-rays in Table 8.8 with Table 8.2, a significantly increased risk of a gastroschisis pregnancy was found for first trimester use of aspirin and for mothers who had a history of gynaecological infection or disease for both analyses. However, the unadjusted odds ratio for the first trimester use of aspirin for the initially selected control analysis was lower than for the replacement control analysis (unadjusted OR 15.58 compared with 19.10). There was also a large increase in the unadjusted odds ratios associated with first trimester exposure to phenylpropanolamine and X-rays (7.24 compared with 2.52

and 4.16 compared with 2.25, respectively), however, both remained non-statistically significant.

Table 8.8: First trimester exposure to prescribed or 'OTC' drugs, X-rays and medical history risk factors. Unadjusted odds ratios for gastroschisis - initially selected controls only compared with all controls.

Exposure present Yes:No		Unadjusted odds ratio	
		Initially selected controls	All controls
First trimester exposure to:			
	aspirin	15.58[a]	19.10[a]
	ibuprofen	1.50	1.37
	antihistamines	1.30	1.50
	phenylpropanolamine	7.24	2.52
	paracetamol	1.02	1.04
	oral contraceptives	2.04	1.92
	anti-depressive drugs	2.62	3.00
	X-rays	4.16	2.25
History of depressive illness		1.83	1.67
History of gynaecological infection/disease[$]		2.73[a]	2.78[a]
Family history of congenital anomalies		1.34	1.31
Parity:	Primiparous	1.63	1.67
	Parity 1	1.00*	1.00*
	Parity 2 or more	1.90	1.93

[a] *Significantly elevated odds in gastroschisis mothers*
[$] *History of chlamydia, recurrent urinary tract infections or abnormal cervical cytology*
** Baseline comparison group*

Once again, a comparison of the initially selected control analysis with the replacement control analysis for the first trimester exposure of mothers to a variety of consumption behaviours indicated similar results (Table 8.9 and Table 8.3, respectively). A significantly increased risk of a gastroschisis pregnancy was found for first trimester cigarette use, the first trimester use of any recreational drug, the first trimester use of any class A or B drug and the first trimester use of ecstasy and cannabis in both analyses. The unadjusted odds ratios for these drugs remained similar except for the use of any class A or B drug where the unadjusted odds ratio was substantially increased in the initially selected controls from 5.76 to 8.53. It was impossible to calculate the unadjusted odds ratio for the first trimester use of heroin in the initially selected control analysis as none of the control mothers reported such use. Mothers who drank 28 or more units of alcohol

Table 8.9: Consumption behaviours during the first trimester. Unadjusted odds ratios for gastroschisis - initially selected controls compared with all controls.

Consumption behaviour Yes:No		Unadjusted odds ratio	
		Initially selected controls	All controls
First trimester caffeine use:	None	1.00*	1.00*
	1 to 9 cups / day	0.79	0.89
	10 to 19 cups / day	0.92	0.95
	20 or more cups / day	1.21	1.49
First trimester cigarette use:	None	1.00*	1.00*
	1 – 10 cigarettes per day	2.26[a]	2.07[a]
	More than 10 cigarettes per day	2.48[a]	2.27[a]
First trimester weekly alcohol consumption:	None	1.00*	1.00*
	Less than 14 units	1.47	1.33
	14 to <28 units	1.84	1.84
	28 or more units	2.43[a]	1.88
First trimester binge drinking:	None	1.00*	1.00*
	6 to 10 units	1.07	1.30
	11 to 20 units	1.08	1.10
	more than 20 units	0.98	1.02
First trimester use of any recreational drug		3.23[a]	3.37[a]
First trimester use of heroin		n/a	18.00[a]
First trimester use of cocaine		5.27	6.00
First trimester use of amphetamine		4.93	4.00
First trimester use of ecstasy		7.81[a]	6.42[a]
First trimester use of cannabis		3.31[a]	3.99[a]
First trimester use of a class A or B drug		8.53[a]	5.76[a]

[a] *Significantly elevated odds in gastroschisis mothers*

per week during their first trimester showed a significantly increased risk of a gastroschisis pregnancy for the initially selected control analysis whereas the increase in the risk associated with this level of alcohol consumption was not statistically significant in the replacement control analysis (unadjusted ORs 2.43 compared with 1.88), however, the actual difference in terms of the size of the unadjusted odds ratio was not large.

8.2.2 Conditional logistic regression analysis – multivariate analysis.

Table 8.10 presents the final conditional logistic regression model for the use of any recreational drug during the first trimester of pregnancy in the gastroschisis mothers using the initially selected controls. The putative risk factors that had a

Table 8.10: Mutually adjusted conditional logistic regression model for the reported use of any recreational drug during the first trimester of pregnancy in Gastroschisis mothers -initially selected controls compared with all controls.

Risk Factor		Adjusted[1] odds ratio	
		Initially selected controls	All controls
First trimester use of any recreational drug:	No	1.00*	1.00*
	Yes	1.78	2.22[a]
Body Mass Index:	Normal	1.00*	1.00*
	Underweight	1.81	2.10[a]
	Overweight	0.26[b]	0.32[b]
	Obese	0.31[b]	0.32[b]
Marital Status:	Married	1.00*	1.00*
	Single / separated / divorced	2.75	1.86[a]
First trimester use of aspirin:	No	1.00*	1.00*
	Yes	14.01[a]	21.39[a]
Home owner:	Yes	1.00*	1.00*
	No	1.76	1.90[a]
History of gynaecological infection or disease:	No	1.00*	1.00*
	Yes	2.48[a]	2.40[a]
First trimester cigarette use:	None	1.00*	1.00*
	1 – 10 cigarettes per day	1.73[a]	1.63[a]
	More than 10 cigarettes per day	2.18[a]	1.85[a]

[1] *adjusted for all other characteristics in the table*
[a] *Significantly elevated odds in gastroschisis mothers*
[b] *Significantly reduced odds in gastroschisis mothers*
**Baseline comparison group*

significant effect upon the conditional logistic regression model using the initially selected controls were identical to those in the replacement control model (Table 8.4). The size of the odds ratios for the initially selected controls were, however, slightly lower with the exception of marital status and a history of gynaecological

infection or disease. After adjusting for these risk factors, although there was an excess risk, of 78% for any reported recreational drug use during the first trimester of pregnancy, in gastroschisis mothers this was no longer significant.

Table 8.11: Mutually adjusted conditional logistic regression model for the reported use of a class A or B drug during the first trimester of pregnancy in Gastroschisis mothers - initially selected controls compared with all controls.

Risk Factor		Adjusted[1] odds ratio	
		Initially selected controls	All controls
First trimester use of a class A or B drug:	No	1.00*	1.00*
	Yes	4.70[a]	3.59[a]
Body Mass Index:	Normal	1.00*	1.00*
	Underweight	1.62	1.99[a]
	Overweight	0.26[b]	0.33[b]
	Obese	0.29[b]	0.30[b]
Marital Status:	Married	1.00*	1.00*
	Single / separated / divorced	2.59[a]	1.79
First trimester use of aspirin:	No	1.00*	1.00*
	Yes	13.39[a]	20.50[a]
Home owner:	Yes	1.00*	1.00*
	No	1.85[a]	2.02[a]
History of gynaecological infection or disease:	No	1.00*	1.00*
	Yes	2.60[a]	2.64[a]
First trimester cigarette use:	None	1.00*	1.00*
	1 – 10 cigarettes per day	1.71[a]	1.62[a]
	More than 10 cigarettes per day	2.03[a]	1.75[a]

[1] *adjusted for all other characteristics in the table*
[a] *Significantly elevated odds in gastroschisis mothers*
[b] *Significantly reduced odds in gastroschisis mothers*
* *Baseline comparison group*

Using the initially selected controls, the risk associated with the reported use of any class A or B drug during the first trimester of pregnancy in the gastroschisis mothers was increased: adjusted OR 4.70 compared with 3.59 (Table 8.11) in the model using replacement controls and adjusting for the same putative risk factors. Once again the same risk factors were included within the model and the size of the odds ratios for the initially selected controls were, however, slightly lower for all factors except marital status and first trimester cigarette use.

Table 8.12: Mutually adjusted conditional logistic regression model for the reported use of cannabis alone, class A or B drugs alone or both cannabis and class A or B drugs during the first trimester of pregnancy in Gastroschisis mothers - initially selected controls compared with all controls.

Risk Factor		Adjusted[1] odds ratio	
		Initially selected controls	All controls
First trimester use of an recreational drug:	No	1.00*	1.00*
	Cannabis	0.83	1.40
	Class A or B drugs	1.20	0.89
	Cannabis and class A or B drugs	14.77[a]	10.86[a]
Body Mass Index:	Normal	1.00*	1.00*
	Underweight	1.69	2.09[a]
	Overweight	0.25[b]	0.33[b]
	Obese	0.29[b]	0.32[b]
Marital Status:	Married	1.00*	1.00*
	Single / separated / divorced	2.62[a]	1.83
First trimester use of aspirin:	No	1.00*	1.00*
	Yes	13.67[a]	20.76[a]
Home owner:	Yes	1.00*	1.00*
	No	1.83[a]	1.87[a]
History of gynaecological infection or disease:	No	1.00*	1.00*
	Yes	2.82[a]	2.71[a]
First trimester cigarette use:	None	1.00*	1.00*
	1 – 10 cigarettes per day	1.73[a]	1.64[a]
	More than 10 cigarettes per day	1.98[a]	1.66

[1] *adjusted for all other characteristics in the table*
[a] *Significantly elevated risk in gastroschisis mothers*
[b] *Significantly reduced risk in gastroschisis mothers*
**Baseline comparison group*

In this third and mutually adjusted conditional logistic regression model (Table 8.12) the risk associated with the reported use of recreational drugs during the first trimester of pregnancy in gastroschisis the mothers is presented as a four level factor. Once again the factors having a significant effect upon the model were the same as in the previous models. The adjusted odds ratio the use of cannabis and class A or B drugs during the first trimester of pregnancy in gastroschisis mothers was significant (adjusted OR 14.77). This compares to a significant adjusted odds ratio of 10.90 in the analysis using replacement controls. Similarly, as in the replacement control model, following adjustment for the other factors neither

cannabis use alone nor use of class A or B drugs alone were significantly associated with being a gastroschisis mother (adjusted OR cannabis 0.83 and class A or B drugs 1.20). Once again the size of the odds ratios for the initially selected controls were, however, slightly lower for all factors except marital status, first trimester cigarette use and reported class A or B drug use.

Model checking for the initially selected control models produced very similar results to the replacement controls.

Results: Part Three – Hair Analysis – validation of the interview data concerning recreational drug use

This chapter presents the results from the hair analysis carried out to validate the data collected at interview from the gastroschisis case and control mothers concerning the use of any recreational drugs during pregnancy.

9.1 Collection and preparation of hair samples

Hair samples were collected from the majority of the gastroschisis case mothers and their controls: 98.6% and 97.9% respectively (Table 9.1). However, GCMS analysis was only carried out for 75.3% of the gastroschisis cases mothers and 58.6% of the control mothers. Although the size of the hair segments for testing was revised after the first 200 samples, from the postulated period of risk for gastroschisis of between 4 and 9 weeks to most of the first trimester, a large proportion of the segments weighed too little for the analysis (ie. less than 5 grams). This affected significantly more of the controls mothers than the gastroschisis case mothers: 28.1% compared to 16.9% (p=0.011). In addition a number of the hair samples were too short for analysis of the period of growth in the first trimester.

Table 9.1: Hair samples and their suitability for hair analysis

Hair analysis	**Gastroschisis cases**		**Controls**		**p value**[a]
	n	%	n	%	
Hair sample provided	142	*98.6*	423	*97.9*	*0.86*
As a proportion of the number of samples collected:					
GCMS carried out	107	*75.3*	248	*58.6*	***0.0005***
Hair sample unsuitable:					
Sample too small (weight)	24	*16.9*	119	*28.1*	***0.011***
Sample too short	9	*6.3*	49	*11.6*	*0.15*
Laboratory problem	2	*1.4*	7	*1.7*	*0.85*

[a] *Chi squared test*

9.2 Hair analysis results

9.2.1 Hair analysis using GCMS to detect the use of recreational drugs.

As described in the methods chapter the GCMS analyses produced a set of results in which there was no overlap between the positive results from maternal interview and the positive results from the hair analysis. A review of the literature had suggested that GCMS was often used in studies as the 'gold-standard' for comparisons as sensitivities of up to100% had been recorded for the most common drugs (Moeller at al. 1992), (Ostrea et al. 2001), (Welp et al. 2003). In this study none of the 18 hair samples that should have tested positive for class A or B drugs from maternal interview, tested positive at GCMS analysis, indicating an extremely low sensitivity. A completely separate group of 18 hair samples were tested positive for class A or B drugs at GCMS. None of these mothers had reported class A or B drug use at interview but in the subsequent RIA and LC-MS/MS analysis seven of these samples were confirmed as positive for class A or B drugs and therefore the specificity of this test, assuming that all the negative results really are negative, was high. These findings are totally contradictory. Although none of the reported drug use was confirmed at GCMS, non-reported drug use was identified at this preliminary analysis. Indeed if the sensitivity of the GCMS analysis was really as low as this first analysis suggested then the fact that seven class A or B drug users were confirmed at RIA and LC-MS/MS analysis would indicate that real levels of class A or B drug use in this population could be unrealistically high.

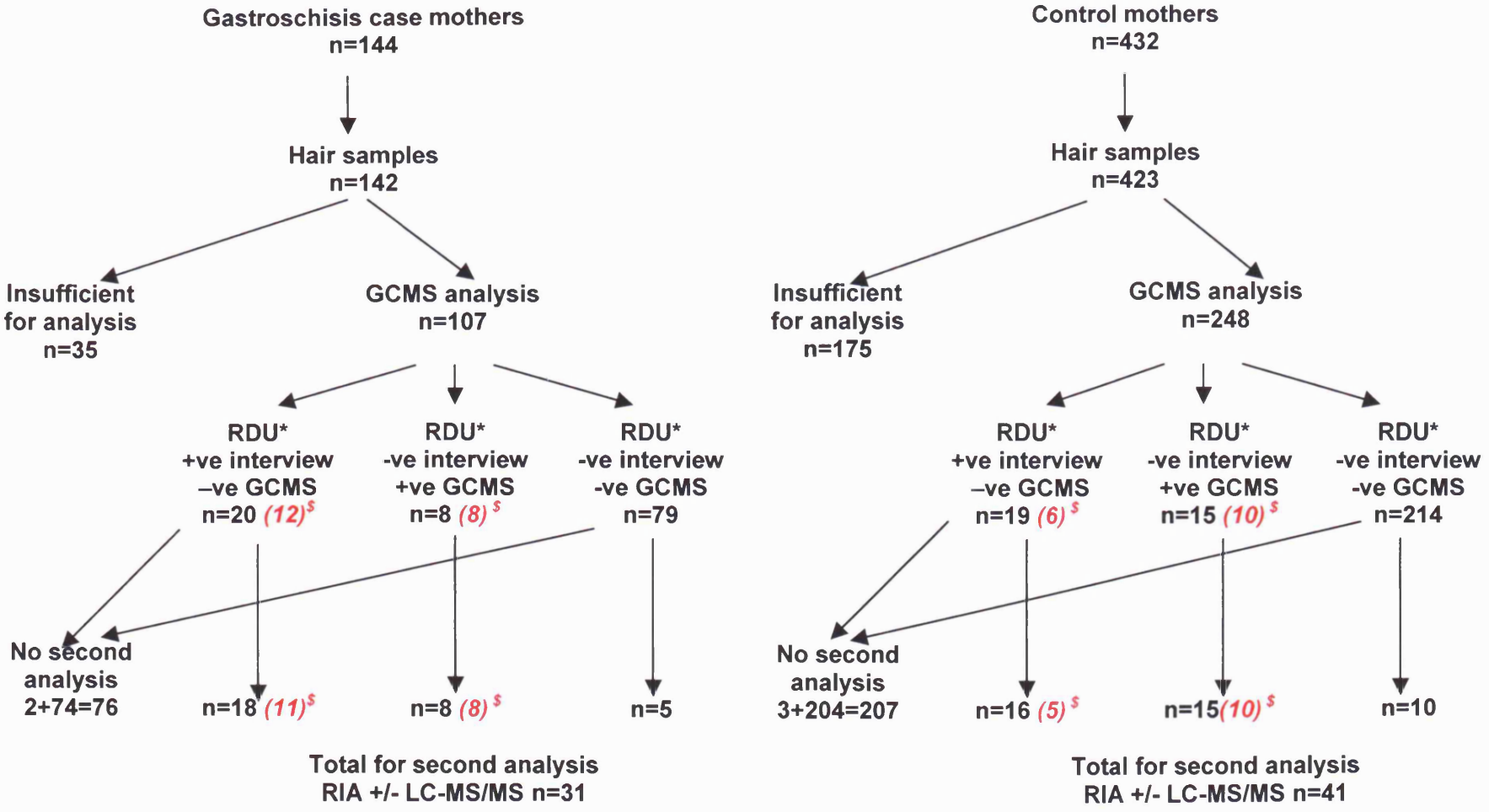
Drug dosage was not collected at maternal interview but information was available concerning the period of pregnancy during which class A or B drug use was consumed. Data from maternal interviews indicated that there was a wide variety of use from just the first few weeks of pregnancy to the whole nine months of pregnancy and the GCMS was unable to detect any of this use. The type of class A or B drug consumed was also investigated to see if any detection pattern could be identified. No obvious pattern emerged. Clearly the pattern of drug use of the class A or B drug users identified from GCMS analysis but negative at maternal interview and confirmed at RIA and LC-MS/MS analysis was unknown.

Due to financial and laboratory time constraints it was possible to reanalyse only a limited number of hair samples. The findings from the GCMS analysis were, at best, contradictory and therefore to try and make some sense of these results the selection of samples for reanalysis was informed by the GCMS analysis results.

9.2.2 Hair analysis using RIA to screen for possible positive results for recreational drug use followed by validation using LC-MS/MS.

Those hair samples where a borderline or positive result for recreational drug use was obtained from the GCMS analysis and those where recreational drug use was reported at maternal interview were analysed using radio-immunoassay (RIA). A small sample (n=15) of hair samples where both GCMS and maternal interview data were negative for recreational drug use were also analysed for a second time. A flow chart indicating the number of case and control hair samples tested at each stage of analysis is presented in Figure 9.1. Two gastroschisis case and three control hair samples where mothers had reported recreational drug use had to be excluded from this RIA analysis as the remaining hair samples were of insufficient weight.

Figure 9.1 Flow chart detailing the number of gastroschisis case and control mothers by each stage of hair analysis



*RDU – recreational drug use
$ positives for class A or B drug use at interview or GCMS analysis

Tables 9.2 and 9.3 show the proportion of hair samples from the gastroschisis case mothers and the controls mothers that were tested at each stage of the hair analysis by their cannabis and class A or B drug status as reported at the maternal interview. Results from the GCMS analysis for class A or B drugs indicated that eight of the gastroschisis mothers had positive hair tests. These are presented in red (Table 9.2) and show that none of the hair samples with positive results from this test were reported as class A or B drug users at maternal interview. Four of these hair samples tested positive for class A or B drugs at LC-MS/MS analysis. In the controls 10 hair samples were found to be positive at GCMS analysis that were not reported at maternal interview (shown in red, Table 9.3). Three of these hair samples tested positive for class A or B drugs at LC-MS/MS analysis.

The results of the RIA analysis and the confirmation of the positive and borderline results using LC-MS/MS are presented in Table 9.4. This hair analysis concentrated on the detection of class A and B drugs. The segment of hair tested for this analysis included the period of conception and the second and third trimesters of pregnancy. Data from maternal interview was used to determine whether it would be possible for the reported recreational drug use to be detected in this analysis. Six gastroschisis case mothers and two control mothers reported using class A or B drugs after the first trimester of pregnancy and all were detected in this analysis. It was not possible to detect any of the other reported class A or B drugs from hair samples, as the first trimester segment had already been used for the GCMS analysis and reported use did not continue into the second and third trimesters. In three of these gastroschisis case mothers and one of the controls the LC-MS/MS analysis detected additional class A or B drugs to those reported at maternal interview.

Table 9.2: Proportion of hair samples from gastroschisis case mothers by their hair analysis test combination status by cannabis and class A/B drug status from maternal interviews, n=142.

Hair analysis test combination*	Status from maternal interview			
	Positive		Negative	
	Cannabis n *(%)*	Class A/B n *(%)*	Cannabis n *(%)*	Class A/B n *(%)*
+ + +	7 (4.9)	6 (4.2)	5 (3.5)	6 (4.4) [4]$
+ + -	8 (5.6)	5 (3.5)	11 (7.7)	14 (9.9) [4]$
+ - -	4 (2.8)	3 (2.1)	72 (50.7)	73 (51.4)
- - -	2 (1.4)	0 (-)	33 (23.2)	35 (24.6)
Total	21 (14.8)	14 (9.8)	121 (85.2)	128 (90.1)

* Test combinations: 1st + = GCMS; 2nd+ = RIA; 3rd+= LC-MS/MS

$ tested positive for class A/B drugs at GCMS

Table 9.3: Proportion of hair samples from control mothers by their hair analysis test combination status by cannabis and class A/B drug status from maternal interviews, n=423.

Hair analysis test combination*	Status from maternal interview			
	Positive		Negative	
	Cannabis n *(%)*	Class A/B n *(%)*	Cannabis n *(%)*	Class A/B n *(%)*
+ + +	2 (0.5)	2 (0.5)	5 (1.2)	5 (1.2) [3]$
+ + -	10 (2.4)	3 (0.7)	24 (5.7)	31 (7.3) [7]$
+ - -	3 (0.7)	2 (0.5)	204 (48.2)	205 (48.5)
- - -	3 (0.7)	1 (0.2)	172 (40.7)	174 (41.1)
Total	18 (4.3)	8 (1.9)	405 (95.7)	415 (98.1)

* Test combinations: 1st + = GCMS; 2nd+ = RIA; 3rd+= LC-MS/MS

$ tested positive at GCMS

Four gastroschisis case mothers and three control mothers who were negative for class A or B drug use from maternal interview but positive at GCMS, had positive results from LC-MS/MS analysis. The additional positive tests all resulted from the hair samples that had been test positive from the GCMS analysis, none were from the small number of retested samples that were both negative at interview and GCMS.

Table 9.4: Validation of maternal interview data for class A and B drug use by RIA and LC-MS/MS

Reanalysis of hair samples	Gastroschisis cases n=142		Controls n=423	
	n	%	n	%
Total samples screened by RIA	31	*21.8*	41	*9.7*
Confirmatory analysis by LC-MS/MS	12	*8.5*	7	*1.7*
As a proportion of the samples reanalysed:				
Negative for class A or B drugs at interview	20	*64.5*	36	*87.8*
Positive for class A or B drugs at LC-MS/MS	4	*12.9*	3	*7.3*
Positive for class A or B drugs at interview	11	*35.5*	5	*12.2*
Possible to detect at LC-MS/MS	6	*19.4*	2	*4.9*
Detected at LC-MS/MS	6	*19.4*	2	*4.9*

There was no working assay available for either the RIA analysis or the accurate determination of cannabis use by LC-MS/MS, therefore the RIA and LC-MS/MS analyses concentrated on class A or B drug use.

Table 9.5: Recreational drug use during the first trimester for gastroschisis case and control mothers – revised with data from LC-MS/MS hair analysis

Recreational drug use	Gastroschisis cases n=144		Controls n=432	
	n	%	n	%
Recreational drug use: None	117	*81.3*	407	*94.2*
Heroin	9	*6.3*	2	*0.5*
Cocaine	4	*2.8*	3	*0.7*
Ecstasy	7	*4.9*	6	*1.4*
Amphetamine	7	*4.9*	5	*1.2*
Cannabis	21	*14.6*	18	*4.2*
Any recreational drug use:	27	*18.7*	25	*5.8*
One drug only	12	*8.3*	19	*4.4*
Two drugs	11	*7.6*	3	*0.7*
Three or more drugs	4	*2.8*	3	*0.7*
Class A or B drug	18	*12.5*	11	*2.5*

The additional results from LC-MS/MS hair analysis were included in the analysis. Table 9.5 presents an overall summary of the recreational drug use of the gastroschisis case mothers and the controls mothers including data from both maternal interview and hair analysis. Gastroschisis case mothers were over three times more likely to have used any recreational drug during the first trimester than control mothers (18.7% vs 5.8%, respectively).

Table 9.6 summarises the data concerning class A or B drug use from maternal interview and hair analysis and provides information about the proportion class A or B drug use that was self reported. Overall, 77.8% of identified class A or B drug use was reported, at interview, by the gastroschisis case mothers and 72.7% by the control mothers. It should be stressed, however that these figures represent the minimum number of possible drug users within this population as only a small proportion of the hair samples were tested using RIA and LC-MS/MS. Further work is being carried out to try and identify any other drug users in this study.

Table 9.6: Method of identification of class A or B drug users by drug type

		Gastroschisis cases		**Controls**	
		n	% *(95%ci)*	n	% *(95%ci)*
Total Class A or B drugs		18	*100.0*	11	*100.0*
Identified at:	Maternal interview	14	*77.8*	8	*72.7*
	Hair analysis	4	*22.2 (6.4 to 47.6)*	3	*27.3 (6.0 to 61.0)*
Proportion of class A or B drugs identified from maternal interview by drug type:					
	Heroin	6 / 9	*66.7*	1 / 2	*50.0*
	Cocaine	2 / 4	*50.0*	1 / 3	*33.3*
	Ecstasy	7 / 7	*100.0*	4 / 6	*66.7*
	Amphetamine	5 / 7	71.4	3 / 5	*60.0*

Details of the specific additional drugs detected at hair analysis are also provided in Table 9.6. Multiple class A or B drug use was detected in one of the gastroschisis case mothers identified at hair analysis with additional drugs being detected for a further four cases who had reported different class A or B drug use

at interview. In the control mothers multiple class A or B drug use was detected in three hair samples where mothers had denied such drug use at interview. In addition, for a further three control mothers, additional class A or B drugs were detected at LC-MS/MS hair analysis, over and above those reported at interview. Once again, however, it should be noted that these findings are an indication of the minimum level of class A or B drug use in the gastroschisis case mothers and their controls.

Details of the use of reported hair preparations (hair dye, bleach or perming solutions) were examined for all the positive results because these are known to reduce the concentration of recreational drugs and their metabolites present in the hair. These are presented in table 9.7. Borderline results were checked for the use of hair preparations. This only affected one gastroschisis case where class A or B drugs had been recorded at maternal interview and a borderline hair analysis result was obtained.

Table 9.7: Positive hair analysis results for class A or B drugs by use of hair preparations.

Source of positive result	Use of hair preparations	Gastroschisis cases		Controls	
		n	%	n	%
Interview & hair analysis	No	4	*40.0*	1	*20.0*
	Yes	2*	*20.0*	1	*20.0*
Hair analysis alone	No	2	*20.0*	1	*20.0*
	Yes	2	*20.0*	2	*40.0*

** one borderline positive result from hair analysis*

Results: Part Four – Attributable risk

This final results chapter presents the estimated attributable risks for gastroschisis associated with the recreational drug exposures and investigates the effect of maternal age on these estimates.

10.1 Estimated attributable risks from maternal interview data

Using the adjusted odds ratios estimated from the analysis including the replacement controls (Tables 8.4 and 8.5) the attributable risks associated with any modifiable behaviours were estimated: recreational drug use, class A or B drug use, cigarette smoking and aspirin use in the first trimester of pregnancy. These are presented in Table 10.1. Prevalence estimates from the matched controls were used in the calculation of the attributable risks associated with each behaviour. The attributable risk of gastroschisis associated with any recreational drug use during the first trimester of pregnancy was 6.1% (95% ci, 1.7 to 19.5) and for class A or B drug use was 4.6% (95% ci, 0.9 to 20.5), this was not statistically significant. Over one quarter of gastroschisis cases were estimated to be attributable to cigarette smoking (estimated attributable risk 26.6%; 95% CI 8.7 to 57.9). Whilst the point estimate of the attributable risk for aspirin was just less than 10% the confidence interval was extremely wide and not statistically significant, due to the very low prevalence of aspirin use during pregnancy (AR 8.6%; 95% ci, 0.1 to 93.8).

Table 10.1 Attributable risks associated with modifiable behaviours reported at maternal interview.

Risk Factor	Adjusted odds ratio*	Prevalence in controls (%)	Attributable risk (%)	95% confidence interval (%)
First trimester use of:				
Any recreational drug	2.22	5.3	6.1	1.7 to 19.5
Class A or B drugs	3.59	1.9	4.6	0.9 to 20.5
Cigarettes	1.73	49.8	26.6	8.7 to 57.9
Aspirin	21.39	0.5	8.6	0.1 to 93.8

** from tables 8.4 and 8.5*

In order to investigate the effect of maternal age on the estimated attributable risk calculations an age-stratified conditional logistic regression model was developed (Table 10.2). The odds ratio was adjusted for the same putative factors as in tables 8.4 and 8.5, with the exception of first trimester aspirin use which was only included in the youngest age group as none of the older control mothers reported taking aspirin. Similarly, it was not possible to calculate the adjusted odds ratio for the first trimester use of a class A or B drug in mothers aged 25 years or more as no controls reported such drug use. The study did not have sufficient power to perform the age-stratified analysis and therefore none of the adjusted odds ratios were statistically significant. In addition these adjusted odds ratios showed no statistically significant differences across the maternal age groups. However, an indication of the possible size of the odds ratio associated with each maternal age group is provided. Mothers who reported any recreational drug use during the first trimester of pregnancy were over twice as likely to have a baby with a gastroschisis (whatever their age) compared to mothers who did not report recreational drug use at this stage of pregnancy. The adjusted odds ratio

Table 10.2: Mutually adjusted conditional logistic regression model for the reported use of any recreational drug during the first trimester of pregnancy, stratified by maternal age.

Risk Factor		Total cases	Cases positive for specified drug use	Adjusted[1] odds ratio	95% confidence interval
First trimester use of any recreational drug:	No			1.00*	
Mothers aged < 20 years:	Yes	58	11	2.02[$]	0.81 to 5.00
Mothers aged 20 to 24 years:	Yes	52	8	2.72	0.82 to 8.97
Mothers aged ≥ 25 years:	Yes	34	5	2.58	0.27 to 24.97
First trimester use of a class A or B drug:	No			1.00*	
Mothers aged < 20 years:	Yes	58	7	2.67[$]	0.76 to 9.39
Mothers aged 20 to 24 years:	Yes	52	5	5.84	0.86 to 39.72
Mothers aged ≥ 25 years:	Yes	34	2	n/a	

[1] *Adjusted for bmi, marital status, home ownership, gynaecological infection or disease & first trimester smoking.*
[$] *Also adjusted for first trimester aspirin use*
* *Baseline comparison group*

associated with such use was highest in those mothers aged 20 to 24 years at 2.72 (95% CI, 0.82 to 8.97). This analysis suggests that mothers aged 20 to 24 years who reported class A or B drug use in the first trimester of pregnancy were nearly six times more likely to have a baby with a gastroschisis than mothers of this age who did not report such drug use. The adjusted odds ratio associated with younger mothers (aged less than 20 years) was much less at 2.67 (95% CI, 0.76 to 9.29) and, as mentioned, it was not possible to calculate this for older mothers aged 25 years or more.

Table 10.3: Age-stratified attributable risk estimates for gastroschisis associated with recreational drug use reported at maternal interview.

Risk Factor	Adjusted odds ratio	Prevalence in controls (%)	Attributable risk (%)	95% confidence interval (%)
First trimester use of any recreational drug:				
Mothers aged < 20 years	2.02	7.5	7.1	1.0 to 36.9
Mothers aged 20 to 24 years	2.72	5.1	8.1	0.8 to 48.2
Mothers aged ≥ 25 years	2.58	2.0	3.0	0.1 to 98.5
First trimester use of class A or B drugs:				
Mothers aged < 20 years	2.67	2.9	4.6	0.4 to 39.0
Mothers aged 20 to 24 years	5.84	1.9	8.5	3.1 to 21.3
Mothers aged ≥ 25 years	n/a	-	-	-

Using the adjusted odds ratio values from Table 10.2 and the estimated age-stratified prevalence of recreational drug use from the control mothers the age-stratified attributable risk associated with any recreational drug use and class A or B drug use was estimated and is presented in Table 10.3. As expected, due to the low prevalence of such drug use in the control mothers and the inadequate power of the study for this age-stratified analysis the 95% confidence intervals associated with the attributable risks at all ages are extremely wide. However, the highest estimated attributable risks of gastroschisis associated with any recreational drug use and class A or B drug use were for mothers aged 20 to 24 years; 8.1% (95% CI, 0.8 to 48.2) and 8.5% (95% CI, 3.1 to 21.3), respectively. The fact that the estimated attributable risk associated with any recreational drug use is lower than for class A or B drug use (a subset of any recreational drug use) is most probably

an indication of the fact that the dataset has been subdivided into too many categories.

Table 10.4 Attributable risk estimates associated with two and three-fold levels of underreporting at maternal interview.

Risk Factor	Adjusted odds ratio*	Prevalence in controls (%)	Attributable risk (%)
First trimester use of any recreational drug assuming:			
One third cases reported	2.22	15.9	16.3
Half cases reported	3.59	10.6	11.5
First trimester use of class A or B drugs assuming:			
One third cases reported	2.22	5.7	12.6
Half cases reported	3.59	3.8	8.8

** from tables 8.4 and 8.5*

Table 10.4 presents attributable risk estimates for gastroschisis associated with first trimester use of any recreational or class A or B drug, assuming a two and three fold level of underreporting by mothers at interview, thus providing an upper estimate of this risk. Gender specific data from the British Crime Survey (Ramsay et al. 2000) indicates similar prevalence levels for non-pregnant women. If only half the mothers who used any recreational drug during the first trimester of pregnancy reported such use at maternal interview then, assuming the odds ratio remains stable, the estimated attributable risk would increase from 6.1% (reported use) to 11.5%, and to 16.3% if only one third of drug users reported their use at interview. Similarly, if only half the mothers who used class A or B drugs during the first trimester of pregnancy reported such use at maternal interview then, once again, assuming the odds ratio remains stable, the estimated attributable risk would increase from 4.6% (reported use) to 8.8%, and to 12.6% if only one third of drug users reported their use at interview. Clearly, estimating the uncertainty of the attributable risk assumes both the adjusted odds ratio and associated 95% confidence interval remain the same, which would inevitably lead to an overestimate of the width of the confidence interval. Therefore the 95% confidence intervals have not been estimated.

Discussion

This project was designed to test the hypothesis that the risk of the abdominal wall defect gastroschisis is positively associated with the use of recreational drugs in the weeks immediately following conception. A matched case control study design was used to test this hypothesis. Maternal hair analysis was used to validate data collected at maternal interview. In this chapter the study design and data validation will be discussed along with their implications for the study findings. This will be followed by discussion and interpretation of the results of the study.

11.1 The study design

11.1.1 Choice of study design

The study design of choice for rare birth defects such as gastroschisis and perinatal epidemiology in general is the case-control study (Clarke and Clayton, 1981), (Torfs et al. 1994), (Werler et al. 1992a). This study design allows for the all the cases within a given population to be studied without the need for the collection of data for the total population of births / pregnancies from that population, as required in a cohort study. The total population of births / pregnancies does, however, act as the sampling frame for the controls in the study. Following the selection of appropriate controls the exposure history for both the cases and controls is collected retrospectively, after the outcome is known, which may lead to bias. This has been discussed at length in the introductory chapter on epidemiological methods in congenital anomaly research. For this study a matched prospective case-control study design was used to test the study hypothesis. The discussion will concentrate on how bias was minimised in this study.

11.1.2 Case definition, identification, selection and recruitment

Clear inclusion and exclusion criteria were defined for the study. All gastroschisis cases were included from the point of diagnosis. This included terminations of pregnancy, late fetal losses, stillbirths and live born infants. The accuracy of the diagnosis was checked following delivery or at post mortem. Other abdominal wall defects and cases of limb-body wall complex were excluded. Over 95% of gastroschisis cases were ante-natally detected and confirmed following delivery. This level of antenatal detection is similar to the highest published detection rates (Fisher et al. 1996); (Kilby et al. 1998). This high level of antenatal detection reduced the effect of any problems of recruitment related to the late diagnosis of a gastroschisis case to a minimum thus reducing any associated selection bias.

This study was established using three congenital anomalies registers with networks of regular notifiers of congenital anomalies already in place around the regions. These registers routinely collect all identified cases of gastroschisis. However, to ensure that all gastroschisis cases were notified to the registers as soon as possible following diagnosis, all relevant health professionals were contacted and provided with information about the study. In addition, a named contact was identified, and regularly contacted by the research interviewers, in fetal medicine clinics, delivery suites, neonatal intensive care units and paediatric surgery units to check for any gastroschisis cases that may have escaped notification to the registers, thus validating registry data. Frequent monitoring of the notification and recruitment of gastroschisis cases to the study was carried out to ensure that all cases were notified to the study office as soon as possible following their detection. Fetal medicine consultants were approached to inform known gastroschisis case mothers at approximately 36 weeks of gestation and ask their permission for one of the research interviewers to approach them for recruitment to the study following delivery. Following some initial problems this antenatal approach was achieved in over 80% of cases indicating that the approach from clinicians was positive and supportive to the study and minimising selection bias.

In this population-based study all cases of gastroschisis were eligible for inclusion over the study period and due to the established networks and regular contact with

all relevant units across the three regions, all resulting gastroschisis cases were notified to the study. Selection bias, for the gastroschisis case mothers, was therefore limited to issues of non-response.

11.1.3 Control definition, selection and recruitment

In order to ensure that the results of a case control study have validity the appropriate selection of the controls is of utmost importance (Rothman and Greenland, 1998). Previous work has shown that about three-quarters of the mothers of gastroschisis cases are less than the age of 25 years (Haddow et al. 1993), (Tan et al. 1996), (Werler et al. 1992a). There is also a clear correlation between the prevalence of recreational drug use and young age (Ramsay et al. 2001), (Schwartz, 1998). Thus, if the control sample had been selected from the general population, because of the relatively low fertility of young women, many of the younger age groups in the control sample would have had very few women. Similarly, within the older age strata there would have been very few cases. It would, therefore, not have been possible to adjust for the potential confounding effects of age in the analysis. To control for the effects of maternal age the control sample was therefore matched to the case mothers by maternal age to within one year (Hennekens and Buring, 1987). As such, matching was used to deal with the potential problem of no overlap at very young or older maternal ages by forcing a directly matched overlap.

In addition to matching on maternal age, controls were matched to cases for region of residence and initial intended place of delivery, mainly for convenience and as a method of defining the sample. The method of selection of controls for this study was developed using personal experience of the selection of controls in perinatal epidemiology (Draper et al. 1999). In many perinatal epidemiological studies control patients are selected from the place of delivery of the cases at the same time for convenience. However, although this minimizes the differential recall by ensuring there is no time lag differential between cases and controls, other bias may be introduced (Clarke and Clayton, 1981). Pregnancies where a congenital anomaly has been detected ante-natally are generally referred to a tertiary centre for delivery. If controls are then selected from tertiary centres they

will, on the whole, be from a high-risk group of mothers and may thus lead to an underestimation of risk (Klebanoff and Rhoads, 1986). By using the initially intended place of delivery of the cases (i.e. the place where mothers were booked for delivery prior to any knowledge of a congenital anomaly) in this study any such institutional selection bias was minimized and a workable method of control selection developed using a network of midwives from around the three regions.

Matching of controls to cases was successful for all three factors. All controls were successfully matched for region of residence and 96.3% of controls were matched for initial intended place of delivery or intended place of delivery of the cases. Less than 1.0% of controls had to be matched for actual place of delivery of the case as either the case (accounting for three of the controls) was never booked for care or the control was not booked for care (one control). The difference in median maternal age between cases and controls was 0.4 years which was not statistically significant.

Whether to use controls with congenital anomalies or 'normal' controls in case-control studies of congenital anomalies remains an issue for debate (Paganni-Hill and Ross, 1982), (Werler et al. 1989), (Lieff et al. 1999b), (Khoury et al. 1994). The use of 'normal controls' in the study was largely pre-determined. This was due to a number of factors. Firstly, the predominantly young age of the mothers of gastroschisis pregnancies meant that there would be few mothers available for matching with pregnancies affected by other anomalies. Secondly, the selection of an appropriate group of anomalies for the comparison group would further complicate their selection and could well have complicated the recruitment process if high levels of antenatal detection of these anomalies did not occur. This would have inevitably reduced the possibility of interviewing the control mothers within the six-week post delivery time window, thus introducing problems of differential recall. Thirdly, if an exposure is related to more than one group of birth defects, then the inclusion of such groups as control subjects may lead to an increased frequency of the exposure in the controls, and thus reduce the size of the resulting odds ratio (Khoury et al. 1994), (Swan et al. 1992). Finally, the organisational complexity of ensuring the rapid notification and recruitment of controls with other congenital anomalies across three health regions would have

been overwhelming. The use of 'normal' controls simplified the recruiting process, allowed for sufficient numbers of maternal age-matched controls to be available and ensured that cases and controls could be selected and interviewed within the same time-frame.

One problem encountered in the recruitment of controls was that, because they were predominantly young women, many stayed with relatives post delivery and were therefore difficult to contact within the six-week post delivery period. As a consequence a network of health visitors was developed after the start of the study to facilitate control recruitment. Successful recruitment of the controls was largely determined by the approach made by the health professional who contacted them about the study. Motivation of a large group of health professionals over a wide geographical area was difficult and it was obvious, from looking at the success levels of recruitment by various individuals, that some were more positive and motivated than others.

11.1.4 Response and the effect of non-response

The response from the gastroschisis case mothers was very high at 87.8%. This is a better response than either of the other two major published case-control studies where responses of between 64% and 79% were reported (Torfs et al. 1994), (Werler et al. 1992a) (Werler et al. 2002). Only 20 of the 164 gastroschisis case mothers did not participate in the study. Fifteen of these were direct refusals; four were associated with lack of patient access either resulting from the patients themselves or by a health professional, and one was notified to the study too late for inclusion. As expected the main difference between responding and non-responding cases was the outcome of the pregnancy. Whilst 45.0% of the non-responding cases were either terminations of pregnancy, stillbirths or deaths these outcomes only accounted for 7.0% of the responding cases. Similarly, 30.0% of the non-responding cases had multiple anomalies compared to only 3.6% of the responding cases. As a consequence of this the gestational age at delivery distribution of the two groups was significantly different. However, there were no statistically significant differences between the non-responding and responding cases in terms of maternal age, plurality, ethnicity or gender.

The major reasons for non-response would therefore appear to be severity of the anomalies and the overall poorer outcomes for this group. The timing of the recruitment of gastroschisis case mothers was carried out at around 36 weeks gestation. For terminations of pregnancy this could obviously not occur and thus this had an effect upon the recruitment of such cases in that there was limited opportunity to recruit some of these mothers as they had little or no subsequent contact with the perinatal services and were often difficult to contact. In three gastroschisis cases with multiple anomalies and/or terminations of pregnancy, access to the mothers was denied by the mother's consultant as they felt that it was inappropriate so close to the delivery, termination or loss. If these mothers were more likely to be recreational drug users then this decision would reduce the estimate of the risk associated with recreational drug use (the main exposure of interest). However, it is unlikely that the decision of a clinician, based on a mother's welfare and leading to non-response, should be directly associated with their use of recreational drugs during pregnancy.

Four hundred and thirty-two controls were required to achieve the full complement of three controls per gastroschisis case. A total of 562 prospective control mothers were approached for this purpose. This represents a response of 76.9% for the women originally approached. Restrictions placed on the study by the multi-centre ethics committee and the Data Protection Act 1998 meant that the only information known about these mothers was the matching variables and therefore a comparison of the characteristics of responders and non-responders could not be carried out. Thus, in order to estimate the effects of potential bias introduced by using replacement controls all analyses were repeated using only those initially selected for each gastroschisis case to overcome any possible criticism of this method of control replacement

As for the cases, the successful recruitment of control subjects was partly due to the level of motivation and support for the study of the allocated midwives and health visitors in the three regions. In instances where specific individuals were noted to have a poor recruitment rate requests were sent to the relevant unit for other study contacts. As epidemiological researchers are only allowed to approach study subjects to ask if they would be willing to participate in a research project, if

they have given their permission via a health professional, the development of a motivated network of health professional contacts was a vital element in this study.

11.1.5 The minimisation of information bias

This study collected information retrospectively. Such data collection is prone to both differential and non-differential recall of exposures. These issues were addressed within the design of the study. In order to reduce non-differential recall to a minimum a structured questionnaire was used to collect information both from maternal notes and face-to-face interview. The recall of medication use and any recreational drug use during pregnancy was facilitated by the use of flash cards, pre-determined prompts and by asking mothers to fetch any medications for the interviewer to see and record. Interviewers went through lists of medical conditions from which the mother may have suffered and/or required medications during pregnancy. Both the official and the street names of recreational drugs were provided on flash cards to check the mother's use during pregnancy. Early pregnancy use of any medication, recreational drug and cigarette, alcohol or caffeine consumption was checked with the mothers by reminding them of the timing of their first month of pregnancy and placing it into context within the year. In addition, it was obvious from early interviews in the study that many of the young women were unaware of the alcohol content of 'alco-pops' and therefore initially reported that they had not consumed alcohol. Fortunately, 'alco-pops' had been included on the prompts check-list for the interviewers and therefore these alcohol users were identified.

A further measure used to minimise recall bias was to interview all study subjects within six weeks of their expected date of delivery. This was aimed at reducing both underreporting due to a large amount of time elapsing between the pregnancy and the interview and eradicating any differential recall due to time differences between the collection of data for cases and controls. The time lag in the other two main case control studies in this area had been considerably longer at around six months post delivery (Torfs et al. 1994), (Werler et al. 1992a).

In this study, both the gastroschisis case mothers and the control mothers reported high levels of smoking during early pregnancy (69.4% and 49.8%,

respectively). However, extrapolation of this apparently accurate reporting cannot be made into other areas, such as the use of recreational drugs, which is an illegal activity. Indeed, many studies have indicated that the prevalence of recreational drug use during pregnancy is under-reported (Shiono et al. 1995), (Kline et al. 1997), (Baumgartner et al. 1989), (Markovic et al. 2000), (Grant et al. 1994) and that a validation method is required to check medical notes and interviewed data.

The lack of validation of data collected from medical notes and maternal interviews has been a limitation of previous case control studies that have investigated the association between recreational drug use and gastroschisis (Torfs et al. 1994), (Werler et al. 2003). A unique aspect of this study was, therefore hair analysis, which was used to overcome this limitation by validating data collected at maternal interview concerning recreational drug use. Results from this analysis were used to estimate the level of underreporting of recreational drug use from the maternal interviews. Previous birth defects research has indicated that mothers having poor perinatal outcomes report adverse exposures more readily than those who have had a successful pregnancy (Werler et al. 1989), (Lieff et al. 1999b). This can lead to differential recall in case control studies where cases with birth defects are compared to 'normal' controls. The hair analysis used in this study was therefore also used to overcome differential recall concerning recreational drug use during early pregnancy.

Although the blinding of both the mothers and the interviewers is recommended in studies (Elwood, 1998) this was not wholly possible in this study for a number of reasons. The information sheet for the study informed the mothers that a small hair sample would be collected to validate the information they provided at interview about substances that they might have been exposed to during pregnancy. A requirement of the Trent MREC was that prescribed medications and recreational drugs were explicitly mentioned on the information sheet. Both the cases and the controls were therefore aware of the fact that their hair was going to be tested for the presence of recreational drugs and were thus, not blinded to the study hypothesis. Despite this virtually all the responding case and control mothers provided a sample for analysis: 98.6% cases and 97.9% controls. However, this information could have been a determining factor in recruitment,

thus leading to selection bias. At least one of the gastroschisis case mothers who refused to participate in the study was a known class A drug user and thus, the study may have under-estimated the risk associated with any recreational drug use and more specifically, class A or B drug use. Replacement controls were selected if the originally selected controls were non-responders and this was an issue for the study.

In order to exclude the possibility that potential bias was introduced by using replacement controls the effect that they had upon the final conditional logistic regression models was examined. Results concerning reported recreational drug use, using the originally selected controls alone, were remarkably similar to the main analysis and if anything, showed a strengthened association between class A or B drug use alone (an increase in the adjusted odds ratio of 1.11) and both cannabis and class A or B drug use (an increase in the adjusted odds ratio of 3.86) despite the reduced power of the analysis. There was, however, a small reduction in the adjusted odds ratio for overall recreational drug use that was no longer statistically significant. The odds of gastroschisis associated with the other putative risk factors in the conditional logistic regression models remained remarkably similar although this was increased in all three models with first trimester cigarette smoking and being single, separated or divorced, and decreased in all three models with first trimester aspirin use and being underweight. Model checking produced similar results to the full analysis. One conclusion of this re-analysis could be that, if anything, the replacement of controls may have reduced the overall risk estimates for both class A or B drug use alone and the multiple use of both cannabis and class A or B drugs as well as reducing the risk associated with first trimester cigarette smoking. However, the reanalysis, using the originally selected controls alone, also incurred the exclusion of six cases, one of whom was a class A or B drug user. It would appear, therefore that the use of replacement control mothers may potentially have led to a small underestimate in the odds of gastroschisis associated with these behaviours. There was no evidence, however, that class A or B drug users and smokers were disproportionately deterred from being recruited to this study, as the proportions of class A or B drug users and smokers in the replacement controls was greater than in the initial controls.

Although much time and effort was spent on the analysis comparing the use of replacement controls with the originally selected controls alone, on reflection, this detailed analysis was probably more extensive than was necessary as there was no evidence that the replacement controls were systematically different from the originally selected controls with respect to the information available. These mothers fulfilled the same matching criteria as the original controls, were approached in the same manner and were given the same information and opportunity to participate in the study. The main conclusions are therefore based on the analysis of all the controls including the replacement controls.

As the study interviewers interviewed the mothers fairly soon after their delivery it was impossible to blind them to the case or control status of the mothers. Furthermore, it would have been inappropriate for them not to be informed of the current status of each infant. In addition, as they were collecting hair samples from the mothers they also were aware of the study hypothesis. However, in order to try and minimise any interviewer bias they underwent a rigorous training. This was to ensure that they used the standardised questionnaire with the flashcards and predetermined prompts in the same manner for all subjects, both cases and controls. Intra and inter-interviewer variation was reduced to a minimum by recording each interviewer interviewing three of the same subjects and comparing their recorded responses and interviewing techniques. Extraction of data from maternal notes was subjected to the same rigorous checks. At the end of each training interview the interviewers rehearsed the collection of maternal hair samples. Interviewers were then accompanied on early visits by the study principal investigator who also repeated the accompanied visits part way through the study. Regular review of the accuracy of each interviewer was checked at the team meetings either interactively within the group or using video review. In the interview situation, following the recruitment, answering of questions and consenting of the mothers, the three interviewers were trained to complete the questionnaire without deviation, asking the mothers to defer any further discussions until the questionnaire had been completed. This ensured that all mothers were interviewed in the trained, standardised manner.

Despite this rigorous training and the emphasis on collecting information in the same manner from both the gastroschisis case and control mothers, less hair was collected from the control mothers. Significantly more of the hair samples from the control mothers were too small for hair analysis to be carried out compared with the gastroschisis case mothers. One explanation for this could be interviewer bias, with interviewers (subconsciously) considering the collection of hair samples to be less important for the controls in general, or more specifically those controls they felt not to be likely recreational drug users. If the controls were felt to be of less importance then the direction of the effect of this bias would be to increase the estimate of the odds of gastroschisis associated with recreational drug use. However, if the interviewers were less likely to collect the hair of those controls that they felt were unlikely to be recreational drug users (and they were accurate in their selection) then this bias would have no effect on the estimate of the odds of gastroschisis associated with recreational drug use. There is unlikely to be an obvious physical reason as to why control mothers had less adequate hair than gastroschisis case mothers.

11.1.6 Data quality

This study involved 576 subjects and collected over 200 separately coded data items for each subject. In order to maintain the highest level of data quality a system of meticulous data checking was carried out. However, inevitably with any large data collection, minor errors may have been missed. Once the questionnaire data had been collected and coded by the three study interviewers the completed questionnaires and hair samples were sent to the main study office. Questionnaires were logged and checked by the study clerk who passed on any outstanding queries for clarification to the principal investigator (ESD). The principal investigator then checked the coding of all data items prior to any input into the Access database. The data entry programme provided basic range checks for variables and checked for any inconsistencies between first and second data entry. Any data coding and validation problems during this process were immediately checked and resolved by the principal investigator, in liaison with the study interviewers if necessary.

Once data entry was complete data cleaning was carried out. Initially range checks of all variables were carried out, including the quantification of any missing data. Use of the double entry Access database had reduced any outliers to a minimum leaving mainly missing data issues to be clarified between the principal investigator and the research interviewers. Logical bi-variate checks (eg. birth weight by gestational age, amount of cigarettes smoked by smokers yes/no, use of appropriate medication with specific illnesses etc.) were carried out and any inconsistencies back-checked against the questionnaires and with the interviewers as necessary. Following this extensive data quality checking by the principal investigator, although there was still the possibility of random errors, there were no errors relating to either case / control status or any of the putative risk factor data.

11.1.7 The power of the study

The size of this study was predetermined by a number of factors: cost, time and the estimated prevalence of gastroschisis. Applications for funding and study time-frames are limited and, as such, it is necessary to design projects to meet these parameters. In order to ensure the greatest number of gastroschisis cases as possible within the three-year total study period and within a limited budget, a collaboration was established between three regional congenital anomaly registers. The number of gastroschisis cases likely to occur over the two and a half years of recruitment for the study was predicted from the then current prevalence recorded by the registers and their annual birth figures. A pragmatic approach was therefore taken to the power calculation as the likely number of possible gastroschisis cases available for the study was known.

When the number of cases for a study is limited the power of a matched case-control study may be increased by the selection of more than one control per case. In general as the ratio of controls to cases increases beyond 4:1 the additional gain in statistical power is small and tends to be outweighed in terms of cost and time (Miettinen, 1969). In this study a ratio of three controls to each case was selected as the benefit, in terms of power, to be gained from a higher ratio of controls appeared to be very small but costly.

At the time of the development of this study there were no UK-wide data available concerning the prevalence of recreational drug use during pregnancy. In fact it was only in 2002 that data from the ALSPAC study (carried out in the early 1990's) was published about the early pregnancy use of cannabis (Fergusson et al, 2002). The estimated prevalence of any recreational drug use during early pregnancy was therefore based on age / gender related estimates from the British Crime Survey (Ramsay & Spiller, 1997) and the Health Education Authority (HEA, 1996) and taken as ten percent. This turned out to be an overestimate as the actual reported prevalence of any drug use in the controls was only 5.3% which had the effect of reducing the overall power of the study.

Using all the information available about the necessary parameters the minimum odds ratio that could be determined from this study was calculated. Setting the power of the study at 80%, with an estimated prevalence of exposure in the controls of 10%, the minimum estimate of the odds ratio that could be detected as statistically significant ($p<0.05$) was 2.5. Ideally it would have been better to design a study with greater power (>90%) with the ability to detect smaller but clinically important odds ratios, but costs both in time and money prohibited this. As a result the study had much less power to investigate sub-groups of the primary outcome.

At the time this study was designed in the late 1990's BINOCAR had not been formalised as a collaborating network of congenital anomalies registers. If this network had been in place it may have been possible to establish a larger study, across all BINOCAR registers, with greater power allowing for the effect of putative risk factors to be investigated further. However, the main costs associated with this type of study are staff costs and if the same methodology was used, with research interviewers visiting all mothers and collecting hair samples, then these costs are likely to have been prohibitive. Of note, it took over two years to secure the funding for this study in its present size.

11.1.8 The conditional logistic regression analysis

The need to ensure that investigations concerning basic biological data about the associations between exposure, outcome and any potential confounders was

highlighted as a limitation of many epidemiological studies in chapter 2. The following strategy for model development was therefore used in this study.

Prior to the development of the stepwise conditional logistic regression model a univariate conditional logistic regression analysis was carried out to identify the factors for inclusion within the model. Once these factors had been identified each one was individually considered in conjunction with the main exposures of interest (any recreational drug use and class A or B drug use) and the outcome, gastroschisis in order to prevent any potential causal associations being included in the adjusted model (Hernan et al. 2002). It was concluded that none of the putative risk factors or confounders were on the causal pathway between the exposure and the outcome and therefore the stepwise conditional logistic regression modelling was performed in the standard manner. However, it was never assumed that the identification of potential confounders or putative risk factors was exhaustive.

11.2 Validation of maternal interview data concerning recreational drug use using maternal hair analysis

11.2.1 Collection and preparation of hair samples

The actual collection of hair samples following maternal interviews proved to be a straight forward process with very few mothers refusing to provide a hair sample for the study: only two (1.4%) gastroschisis case mothers and nine (2.1%) control mothers (no significant difference between cases and controls p=0.86). Samples were stored in sealed plastic bags and marked with study identifiers. On receipt in the research office the samples were relabelled with a random number and measurements indicating the segment for analysis. Hair samples were sent to the chemical pathology laboratory for analysis in batches of 100 samples.

Strict washing protocols were followed for all hair samples and the solutions left over from hair washing were analysed for contaminants. This ensured that any positive findings from the hair analysis were not due to the external contamination of the hair with recreational drugs or their metabolites.

11.2.2 Hair analysis using GCMS and associated problems

All hair analysis for the study was carried out in collaboration with the University Department of Chemical Pathology. However, during the development of the study, advice and general direction for the establishment of the hair analysis had been provided by a member of the University Hospitals of Leicester (UHL) Chemical Pathology Department who worked closely with the university department. This member of staff wanted to develop the local department into a centre of excellence for hair analysis and thus arranged for an MSc project to be carried out to develop the techniques and preparation protocols. Unfortunately, by the last year of the study (2003), the period when it was planned to carry out the hair analysis, this staff member no longer worked for the UHL Chemical Pathology Department and problems were encountered with the organisation of the hair analysis and the supervision of the technician employed to carry out the work. Thus, preparations within the departments had not been made to allow for the hair analysis to take place and basic issues such as the purchase of a ball grinder to grind the hair samples and an application for a Home Office licence to store standards for the recreational drugs had not been set in place. Following regular meetings between the principal investigator, members of the project team and the University and UHL Chemical Pathology Departments these problems were eventually, although tardily, resolved.

By early September 2003, however, it was obvious that there were still problems. Although 400 hair samples had been sent to the laboratory only 200 hair samples had been washed and ground for analysis. None had, at this point, been analysed as the training of the technician had not been completed and adequate staff were not available to supervise the analysis and interpret the results. Following many more hours of negotiations the UHL Chemical Pathology Department agreed, in November 2003, to release their trained member of staff to work on this project both supervising the technician and carrying out the GCMS hair analysis and interpreting the results.

In mid November 2003 the principal investigator was advised that many of the hair samples were of inadequate weight for analysis. Hair samples were sent to the laboratory marked with a random number for identification purposes and measurements indicating the segment of hair for analysis i.e. the segment growing at the suspected aetiological time window. Adjustment to the size of the segments was therefore required to ensure that the majority of the samples could be tested. First trimester segment measurements were therefore calculated. This was the only way to deal with this problem as the study recruitment and interviewing was virtually finished and therefore the collection of larger hair samples from the mothers could not be carried out.

Unfortunately this was not the end to the hair analysis problems. Four months after the official end of the study contract (April 2004) the GCMS results were finally completed. Comparison of these results against the data collected at maternal interview immediately indicated that there was a major flaw in this analysis. A number of study subjects were known (from both their medical records and self-report) to be class A or B drug users and their results were negative at GCMS analysis. Indeed there was no overlap between the results from the GCMS analysis and the maternal interview data. After detailed scrutiny of the laboratory log-book, excel spread sheets and all other available information there was no evidence of systematic errors, mixing up of the hair samples and their allocated random numbers and no possibility of a logical solution. It was concluded therefore that the problem lay with the GCMS analysis. This was, however, completely contrary to the evidence from the literature concerning the sensitivity of GCMS. Previous studies suggest that the sensitivity of GCMS hair analysis for the detection of recreational drugs is very high, with some studies reporting sensitivities of 100% for a variety of drugs including cocaine, opiates and cannabis (Ostrea, 2001) (Welp et al. 2003). However, given the complete lack of overlap between maternal report and GCMS results and following extensive discussions with the laboratory staff it was concluded that a further analysis of the hair samples of mothers, who had reported recreational drug use during pregnancy, was necessary. In addition, an investigation of whether the positive results from the GCMS analysis were, in fact, true or false was required, as the specificity of these tests is difficult to calculate, there being no real 'gold standard'. In an effort to

rescue this aspect of the study a second round of hair analysis was agreed with the UHL Department of Chemical Pathology. Three groups of hair samples were selected for reanalysis:

(i) where a positive result for recreational drugs was found by the GCMS analysis – to determine whether these were true positives;
(ii) where a positive result for recreational drugs was anticipated from the maternal interviews – to test whether the method of hair analysis could pick up actual recreational drug users; and
(iii) for a small sample (n=15) of hair samples that were negative for recreational drugs from the GCMS analysis and maternal interview data – to determine whether these were true negative results.

The number of samples tested from group (iii) was limited due to the costs and time available for this re-analysis. Further work, re-analysing 100 more hair samples that were negative from both maternal interview and GCMS analysis will be carried out to validate the negative status of these hair samples.

In summary, major problems were encountered with the GCMS analysis. As no logical explanation could be found to explain the complete lack of agreement between the maternal interview data and the results of the GCMS analysis a secondary analysis was planned to try and salvage this aspect of the study. The rationale behind the selection of hair samples for the second analysis was to validate, where possible, positive results from the hair analysis and to retest all positive results from the GCMS analysis. One extra level of validation was to include a small sample of hair samples with negative results from both maternal interview and GCMS to check their negative status.

11.2.3 Hair analysis using Radio-immunoassay and validated with Liquid Chromatography tandem Mass Spectrometry

Segments of hair from the appropriate time window had already been used for the GCMS analysis and therefore hair segments were selected from around sample one i.e. one centimetre from around the time of conception and the remaining hair

that was growing during pregnancy. Information about the use of recreational drugs was collected for the whole pregnancy in the maternal interview and therefore this was used to validate the remaining information. These three groups of hair samples were screened for the detection of recreational drugs using radio-immunoassay (RIA). Results from screening by RIA fell into three categories: negative, borderline positive and positive. All 15 hair samples that were negative from maternal interview and GCMS analysis were validated as negative at the RIA screen. However, although this was initially felt to be reassuring (in that no false negative results had been found) as this was a tiny sample, the likelihood of a positive result from 15 hair samples, where the prevalence of recreational drug use, for gastroschisis and control mothers combined, was less than 10% , was only small, one or two samples at most. As such, it cannot be assumed that the negative findings from these samples applied to the rest of the samples that were negative for recreational drugs at GCMS and maternal interview. The lack of retesting of a greater number of these hair samples is therefore a major limitation for this study and further work is being carried out to address this outwith the results presented in this thesis.

All borderline positive and positive results were then validated using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS see Appendix H). The LC-MS/MS analysis focused on class A and B drugs alone as the laboratory had no assay available for the detection of cannabinoids. Validation of cannabis use was not therefore possible.

Maternal interview data was used to determine whether, with the remaining limited hair samples, it would be possible to pick up known class A or B drug users from the LC-MS/MS analysis. Interview data indicated that eight mothers whose hair samples were re-analysed had used class A or B drugs at the time of conception and/or after the first trimester pregnancy, and of these all eight had positive results from the LC-MS/MS analysis. In addition the LC-MS/MS analysis confirmed positive results for class A or B drug use in seven of the hair samples that had previously tested positive at GCMS.

Overall this second hair analysis successfully confirmed all possible users of class or B drugs identified at maternal interview and the negative status of the small

sample of subjects where both maternal interview and GCMS analysis had proved negative for class A or B drug use. In addition a small sample of subjects were identified as positive for class A or B drug use who had initially been identified as negative from maternal interview but positive from the GCMS analysis. An assumption was made that these subjects had used these class A or B drugs in early pregnancy as the hair sample covered the time of conception and the rest of pregnancy and none of the other reported drug users in the study had taken up using recreational drugs part way through pregnancy i.e. they had been using drugs at the beginning of pregnancy and had either continued throughout or given up at some point during pregnancy.

11.2.4 Hair analysis results

Hair samples were provided for over 98% of all subjects in this study (98.6% of gastroschisis case mothers and 97.9% of control mothers). However, significantly more of the gastroschisis mother's hair samples were suitable for analysis than the control mother's hair samples: 75.3% compared to 58.6% (*p=0.0005*). The main reason for this was because many of the samples were too small (i.e. weighed less than 5 mg). There were also more samples where the hair was too short or where laboratory problems occurred in the control mothers but these differences were not significantly different from the gastroschisis case mothers. Hair samples were allocated a random number and sent to the laboratory. As such the case or control status of the sample could not be determined and bias could not have been introduced from such knowledge. It is unclear why significantly more control mothers had insufficient hair for analysis. As discussed in section 11.1.5 one reason could be interviewer bias.

Hair preparations are known to have an effect upon the detection of recreational drugs and their metabolites at hair analysis (Jurado et al. 1997), (Potsch and Skopp, 1996). Information about the use of hair preparations including hair dye and perming solutions was therefore collected at maternal interview. The status of hair preparation use was to be used to help determine the positive status of borderline positive results. However, this situation only occurred once, in a gastroschisis case mother who had admitted to the use of class A or B drugs at

maternal interview; a result already counted as positive. The lack of any further borderline positive results associated with or without the use of hair preparations suggests that any further class A or B drug use in the gastroschisis case or control mothers was occasional or infrequent and not detectable by the RIA analysis.

The RIA screening followed by LC-MS/MS analysis confirmed the presence of class A or B drugs in the hair samples of 2.7% (n=4) of gastroschisis case mothers and 0.7% (n=3) of control mothers. Estimations of the actual level of the underreporting of recreational drugs by both case and control mothers are discussed in section 11.3.4.

11.2.5 Implications for future work

Although the collection of hair samples proved to be acceptable and straight-forward the adequacy of the samples collected varied between subjects. If future studies want to focus on a very short time period and as such, a segment of hair measuring only 1 to 2 centimetres then the size of sample to be collected has to reflect this, consequently being fairly large. This will have an effect upon the acceptability of hair collection for study subjects. The collection of 100 hairs from a subject with lots of thick hair does not present any problems as a discrete sample of hair from the posterior vertex section of the head is easily disguised with the remaining hair. However, in subjects with fine and/or sparse hair the collection of 100 or more hairs may not be acceptable and indeed, even if this many were collected, may still not be of an adequate weight for analysis when focusing on one small segment of the hair. In the forefront of hair analysis techniques are being developed where recreational drugs and/or their metabolites can be detected in as little as one hair (Wainhaus et al. 1998). In studies focusing on short time periods, using small segments of hair, these new techniques should be considered.

The main problem for this study was that hair analysis was not an established procedure within the Chemical Pathology laboratory and had to be set up from scratch for this project. If the staff member, who had been involved with this project from its inception, had not left prior to the establishment of the hair analysis

in the laboratory, then this would not have been a problem. However, since this was the case the study was held up whilst this new process was established within the laboratory. Although preliminary procedures had been established and a validation of the technique carried out, as part of a master's degree, the GCMS analysis did not work in practice. The laboratory is investigating the reasons for this failure. One major factor was the lack of experienced personnel within the department. However, for any future project the experiences of this study recommend the use of a laboratory with an established routine hair analysis programme with evidence of high levels of the sensitivity of the analysis. An adequate budget should be allocated for this purpose as this analysis is likely to be expensive.

Despite the problems with the hair analysis validation of interviewed data using RIA as a preliminary screen and validating any positive or borderline positive findings using LC-MS/MS was carried out successfully for class A and B drugs. However there were problems with the RIA screen for cannabis and the laboratory did not have an assay for validation purposes of cannabinoids at LC-MS/MS. This issue would need to be rectified for any further work in this area.

This study limited the hair analysis to three levels of recreational drug use: none, borderline positive or positive. Validation of interviewed data for class A or B drug users confirmed their use in all possible subjects and a number of additional subjects were confirmed as being class A or B drug users. However, although definite class A or B drug users were confirmed using hair analysis this study was not able to predict the number of 'one off', infrequent or sporadic recreational drug users who did not admit to such use at interview and were not picked up by the hair analysis. This study has most probably therefore underestimated the overall proportions of class A or B drug users by missing 'one off' or very rare use.

11.3 The main findings of the study

11.3.1 Prevalence and epidemiology of gastroschisis

The overall prevalence of gastroschisis in this study (livebirths, fetal deaths and induced abortions) was 4.24 per 10,000 total births. Compared to data from the larger EUROCAT registries from 2000 to 2002 this study prevalence is amongst the highest rates: which range from 0.25 per 10,000 total births in Tuscany, Italy to 4.21 in Paris, showing an increasing gradient from southern to northern Europe. The overall rate for all full member EUROCAT registries was 2.00 per 10,000 total births (www.eurocat.ulster.ac.uk) over this period. This data is collected in a standardised manner across Europe and includes all live births, fetal deaths and induced abortions (EUROCAT, 2002). However, although the routine ascertainment of the registries is good for major anomalies, such as gastroschisis, the additional efforts in special studies, to ensure complete data collection, inevitably lead to complete ascertainment and a consequential higher (if only minimally) prevalence.

The main characteristics of the gastroschisis cases and their mothers in this study were very similar to published data. The median age of all the gastroschisis mothers for this study was 20.7 years, very similar to the median age reported by NCAS over the period 1987 to 1993 (Tan et al. 1996). In the literature the quoted median gestational age for gastroschisis cases is 37 weeks (Tan et al. 1996), (Dillon and Renwick, 1995), (Adra et al. 1996), (Torfs et al. 1994) compared to 36 weeks in this study. As there is still some debate about the best mode of delivery for gastroschisis cases up to half of all such cases are delivered by caesarean section (Sakala et al. 1993), (Blakelock et al. 1997), (Lewis et al. 1990), (Rinehart et al. 1999), this compares to 43.1% in this study. The reported rates of gastroschisis cases with multiple anomalies range from 6% to 25% (Morrow et al. 1993), (Fisher et al. 1996), (Torfs et al. 1990), (Roberts and Burge, 1990). Over the period of this study in the three health regions only 6.7% of gastroschisis cases had multiple anomalies over and above those associated with the condition

which is at the low end of the published reported rates. Inclusion of both associated anomalies and all others in this study gives a rate of 15.2%: in the middle of the quoted rates. There was a very high survival rate of 88.5% at one year of age for the total sample of gastroschisis cases within this study. This compares favourably with studies where survival at one year of age has been found to be around 90% (Stringer and Mason, 1997), (Tawil and Gillam, 1995).

11.3.2 Prevalence of consumption behaviours in the mothers of gastroschisis cases and their matched controls

A major aspect of this study was the collection of information about consumption behaviours at maternal interview. Data collection for the study included the main behaviour of interest, recreational drug use, as well as cigarette smoking, alcohol and caffeine consumption. High levels of cigarette smoking during the first trimester of pregnancy were found in both the cases and controls in this study: 69.4% in the gastroschisis case mothers and 49.8% (47.5% in the originally selected controls) in the control mothers. One factor to account for this is that over three-quarters of these mothers were under the age of 25 years and therefore high levels of smoking would be anticipated. These reported levels of smoking during pregnancy were higher than those reported in the two major US cases control studies of gastroschisis (Torfs et al. 1994), (Werler et al. 1992a), (Werler et al. 2003). However, since these US studies were not maternal age matched and there are major cultural differences between the UK and USA these rates are not directly comparable. Data from the Leicestershire Perinatal Mortality Survey (PMS) random sample of control mothers (Draper et al. 1999) indicate that levels of smoking in mothers under the age of 25 years were 44.4% over the period 2000 to 2002 (unpublished data). In addition, data from the Infant Feeding Survey in 2000 (Hamlyn et al. 2002) indicated that although only 19% of women continued to smoke throughout pregnancy, the proportion of smokers in teenage pregnant women was 39%. It would appear, therefore, that the prevalence of smoking in the control mothers for this study was slightly higher at 49.8% (47.5% originally selected controls).

Similarly, at first sight, reported alcohol consumption levels were high in this study with 68.8% of gastroschisis mothers and 60.0% (61.0% of the originally selected controls) of control mothers reporting first trimester alcohol consumption. Once again this reflects the overall young age of the mother in the study. Comparative data from the Leicestershire Perinatal Mortality Study random sample of controls indicates that a similar proportion (50.8%) of mothers under the age of 25 years reported first trimester alcohol consumption over the period 2000 to 2002 (unpublished data). However, once again the reported levels of alcohol consumption from this gastroschisis study are higher than in other work in this area (Torfs et al. 1994), (Werler et al. 1992a), (Werler et al. 2003) but these rates are not directly comparable.

There is little self-report data available about the prevalence of recreational drug use during pregnancy. Data from the Avon Longitudinal Study of Parents and Children (ALSPAC) indicates that 2.6% of pregnant women across all ages reported using cannabis in the first trimester of pregnancy (Fergusson et al. 2002), whilst in a Dublin study of pregnant women, urinary analysis (without interview data) following the first antenatal appointment only detected 1% cannabis use (Bosio et al. 1997). However, the median age of these mothers was significantly higher, than in this gastroschisis study, at 27 years compared to 20.9 years. Additionally, urinary analysis is limited to picking up substances used within the last 3 days. Mothers, knowing that they may have a variety of tests at booking for antenatal care, may well abstain from the use of recreational drugs prior to their hospital appointment. Cannabis use in this gastroschisis study was reported by 4.2% of control mothers, indicating, once again, that reporting levels for consumption behaviours were higher for this younger group of women. Overall recreational drug use, detected from urinary analysis in the Dublin study (Bosio et al. 1997) was 3.6% compared to 5.5% of the control mothers in this gastroschisis study.

Overall, there is little data available, from the UK, concerning consumption behaviours in young pregnant women for comparison. Comparison of the prevalence of consumption behaviours from the controls in this study with the data

available suggests that these young pregnant women reported a high prevalence of all these behaviours.

11.3.3 Suggested risk factors for gastroschisis from the univariate analysis

The descriptive analysis of the case control data provided a focus for the conditional logistic regression analysis. As a result, unadjusted odds ratios were calculated for three main groups of risk factors for gastroschisis. Firstly, a number of socio-demographic characteristics were reviewed. Overall, the gastroschisis mothers appeared to be more socially deprived than their matched controls. Mothers who were single, separated or divorced (compared with those who were married) and those who did not own their own home (compared with homeowners) had an approximately two and a half times the risk of a gastroschisis case and unemployed mothers had nearly twice the risk of a gastroschisis case compared with those of social class I, II or III non manual. However, financially unsupported mothers had a reduced risk of gastroschisis. Although social status was measured in different ways in the CBDMP case-control studies of gastroschisis, i.e. in terms of annual family income, overall findings were similar (Torfs et al. 1994) in that those mothers who had the lowest incomes appeared to have the highest risk of gastroschisis. In addition, unsupported mothers were also found to have no excess risk of gastroschisis (Torfs et al. 1994). Werler at al (1992a) reported very little information about socio-demographic factors for comparison.

An inverse relationship was found between the maternal body mass index and the risk of gastroschisis. Although the odds ratio associated with mothers who were defined as underweight compared with mothers of normal body weight failed to reach statistical significance there was an indication of a 70% increased risk. Conversely, there was a decreasing risk of gastroschisis associated with mother's increasing weight with an odds ratio of approximately one third for overweight mothers and one quarter for obese mothers. Lam and colleagues (1999) found a similar relationship although the odds ratio associated with being underweight was over threefold and statistically significant in their study.

The second group of risk factors for gastroschisis in this study were medical factors including first trimester consumption of prescribed or 'over the counter' medications, first trimester exposure to X-rays and any medical history factors. Werler et al (1992b) were the first group to suggest an association between gastroschisis and the use of salicylates (aspirin), though their finding was only of borderline significance (Werler et al. 1992b). A threefold statistically significant risk associated with salicylate use was subsequently reported in Spain (Martinez-Frias et al. 1997) followed by an even higher risk (odds ratio 4.67) reported from the CBDMP (Torfs et al. 1996). In the study reported here, although very few women took aspirin in the first trimester, the unadjusted odds ratio of gastroschisis associated with aspirin use was nineteen fold. Significant associations between gastroschisis and various other medications have also been found including acetaminophen (paracetamol) (Werler et al. 1992b), phenylpropanolamine and ibuprofen (Torfs et al. 1996) and also exposure to X-rays (Torfs et al. 1996). Although the odds ratios for these factors were raised in this study none of them were statistically significant and could, therefore, have been chance findings. Women reporting analgesic/antipyretic drug use rarely reported an associated influenza or other underlying illness which could have accounted for the raised odds ratios. However, mothers with a history of a gynaecological infection or disease (defined as recurrent urinary tract infections, chlamydia or abnormal smears) had over two and a half times the risk of a gastroschisis pregnancy (which was statistically significant) adding a further dimension to the characteristics of women at an increased risk of this anomaly.

The third group of risk factors considered in the univariate analysis were consumption behaviours, including the main factor of interest recreational drug use. Although a dose-response relationship was found with increasing consumption of caffeine there was no significantly increased risk of gastroschisis associated with caffeine consumption, confirming other work in this area (Werler et al. 1992a). However, despite previous work showing a significantly increased risk of gastroschisis with alcohol consumption and binge drinking (Werler et al. 1992a), (Torfs et al. 1994) this finding was not duplicated in this study. An increasing risk of gastroschisis was found with increasing alcohol consumption but this finding failed to reach statistical significance. Additionally the study failed to

show any dose-response relationship with increased binge drinking. Both cigarette smoking (Goldbaum et al. 1990), (Haddow et al. 1993), (Martinez-Frias et al. 1997), (Werler et al. 1992a), (Torfs et al. 1994), (Drongowski et al. 1991) and recreational drug use have been linked with an increased risk of gastroschisis (Torfs et al. 1994), (Martinez-Frias et al. 1997), (Drongowski et al. 1991) and are known to have a vaso-constrictive effect upon the developing fetal circulation (Hoyme et al. 1983), (Van Allen, 1981). A two-fold statistically significantly increased risk of gastroschisis was found to be associated with cigarette smoking in this study, similar to recent work in Boston, USA (Werler et al. 2003) though somewhat higher than other reported findings (Werler et al. 1992a), (Torfs et al. 1994). Increased unadjusted odds ratios for gastroschisis were found for all recreational drugs although, as in other work (Torfs et al. 1994), these were only found to be statistically significant for some groups. In this study these were the reported use of any recreational drug, and specifically, the use of a class A or B drug, heroin, ecstasy or cannabis.

11.3.4 Significant findings from the multivariate analysis

Three final models were produced focusing on different aspects of reported recreational drug use: any recreational drug use compared to no recreational drug use, class A or B use compared to no class A or B drug use and a four level factor, no drug use, use of cannabis only, use of class A or B drugs only and use of both cannabis and class A or B drugs. The same group of factors had a significant effect upon the fit of the three final conditional logistic regression models. These factors were: body mass index (BMI), marital status, first trimester aspirin use, non homeowner, history of gynaecological disease or infection and cigarette smoking. These factors are remarkably similar to other gastroschisis case control studies (Torfs et al. 1994), (Werler et al. 2003) with the exception of BMI and history of gynaecological infection. Body mass index has been considered as a risk factor for gastroschisis in conjunction with aspirin use, dietary intake and other pregnancy factors but not with recreational drug use (Lam et al. 1999). However, history of gynaecological disease or infection has not been considered as a factor in other studies. Having a history of gynaecological infection or disease adds another dimension to the description of the lifestyle of mothers who are at risk of a

gastroschisis pregnancy. Many of these mothers are very young and socially deprived. In addition the prevalence of cigarette smoking within this group is very high and approximately one fifth of these mothers use recreational drugs. It is therefore not unlikely that these young women are also in a high-risk group for sexually related diseases such as chlamydia and abnormal cervical cytology (Baguley, 2002).

The adjusted odds ratio for gastroschisis associated with any reported recreational drug use in this study was over two-fold and three and a half for class A or B drug use. These findings confirm other work concerning recreational drug use and the risk of gastroschisis (Werler et al. 2003), (Torfs et al. 1994). Positive findings from the hair analysis for class A or B drug use suggest that at least 2.7% (n=4) of gastroschisis case mothers and 0.7% (n=3) of control mothers did not admit to using such drugs at interview. The odds of gastroschisis associated with recreational drug use would therefore be expected to increase if these data were added to the conditional logistic regression model. However, only 9.7% of hair samples from the control mothers were tested using the RIA and LC-MS/MS compared to 22.0% in the gastroschisis cases and could account for the differential 'pick-up' rate. In terms of the total number of cases and controls the proportion of drug users identified by the hair analysis is small. Conversly, as an overall proportion of identified drug users (22.2% of gastroschisis case mothers and 27.3% of control mothers) did not admit to their recreational drug use at maternal interview, a substantial level of underreporting is suggested. Given the problems with the hair analysis and the need for additional confirmation of the maternal interview and GCMS negative hair samples, this is almost certainly an underestimate of the true level of underreporting. Other studies of recreational drug use during pregnancy have suggested higher levels of underreporting (Bosio et al. 1997), (Shiono et al. 1995), (Kline et al. 1997). It is possible, nonetheless, that because the mothers were aware that hair samples were being analysed for the presence of recreational drugs, they were more likely to be truthful about their use of recreational drugs.

There were slightly, but not significantly, higher levels of underreporting, identified at hair analysis, in the controls mothers compared to the gastroschisis case

mothers (Table 9.6). In addition there was a lower proportion of controls' hair samples sent for RIA and LC-MS/MS analysis. If more control hair samples had been analysed then the likelihood of finding more positive results would be increased thus reducing the odds of gastroschisis associated with class A or B drug use. However, if the GCMS analysis, despite all the problems, identified the majority of samples where mothers had taken drugs but not reported their use at interview, then very few additional positive samples would be identified, any differential underreporting between cases and controls would remain minimal and there would be little effect on the odds of gastroschisis associated with class A or B drug use. Overall, it is likely that, if anything, slightly more underreporting in the control mothers has led to a small overestimate of the odds of gastroschisis associated with class A or B drug use in the first trimester of pregnancy.

It is unlikely that all class A or B drug users have been identified, especially those who only took a class A or B drug occasionally or just once during early pregnancy. This would only have an effect upon the estimate of the odds of gastroschisis associated with class A or B drug use if there was a differential identification of occasional use between the cases and the controls. There is, however, no evidence to suggest that this situation occurred. A further problem was that it was not possible, in this study, to determine the level of underreporting for cannabis use. Unfortunately there is very little data available concerning the use of recreational drugs during pregnancy in the UK. As previously mentioned the only study to report such data is the ALSPAC study and this was limited to cannabis use alone, reported as 2.6% prevalence, for mothers of across all ages, in early pregnancy (Fergusson et al. 2002). Data concerning the recreational drug use of young non-pregnant women indicate that levels of reported recreational drug use in the last month may be as high as 12% in those under the age of 20 years, 16% in the 20 to 24 year age group and 6% in those of 25 to 29 years (Ramsay et al. 2000). Given the risk factor profile of the mothers within this study and the fact that the majority will not have planned their pregnancy, these recreational drug rates may provide a more accurate estimate of the underreporting levels. If this is the case then the actual levels of recreational drug use may be between two and threefold greater than the reported levels.

The power of this study was calculated assuming an exposure to recreational drugs in the control group of 10% (Ramsay et al. 2001), in order to detect an odds ratio of 2.5 at the 5% level of statistical significance, with 80% power. The highest predicted number of gastroschisis cases used in this calculation was 118. The study actually recruited 144 gastroschisis case mothers. However, the reported prevalence of any recreational drug use in the control mothers was only 5.5%. Despite this both the models for any recreational drug use and class A or B drug use had significantly raised odds ratios for gastroschisis, and model checking indicated that these models were robust. Nevertheless, unsurprisingly, model checking indicated that the four level model for recreational drug use was not robust due to a lack of adequate power within the study as there were too few cases and controls within some of the levels of recreational drug use. Adjusted odds ratios for gastroschisis from the final four level model suggested an increasing trend with increasing drug use i.e. from cannabis to the use of both cannabis and class A or B drugs. This warrants further investigation but would require a large study to allow for the low prevalence of polydrug use in young pregnant women.

Every effort was made to collect information about the known risk factors for gastroschisis. However, environmental issues have not been considered eg. proximity to landfill sites (a suggested confounding factor for gastroschisis), (Dolk et al. 1998), (Fielder et al. 2000), as these data were not readily available. It is also possible that there are, as yet, other unknown confounding factors.

11.3.5 Attributable risk

The attributable risk calculations carried out in this study assume that the exposure to the risk factor in the control group is a proxy for the population at risk. In matched case control studies this may not be the case as the control population is not necessarily representative of the general population. This study provides an extreme example of this where the cases are highly skewed with respect to younger maternal age and the controls matched on maternal age, are similarly highly skewed. The controls are, therefore, not at all representative of the general population of births in terms of maternal age. As such age stratified attributable

risk estimates have been calculated and indicate the size of the attributable risk of gastroschisis associated with first trimester recreational and class A or B drug use by maternal age. Unfortunately this study did not have adequate power for this analysis and therefore these estimated attributable risks should be treated with extreme caution. Additionally the calculation of the 95% confidence intervals around an attributable risk estimate can be complex (Greenland, 1989). In this study a logit transformation method was used to calculate the 95% confidence intervals as such estimates are not normally distributed, thus ensuring a positive value for the lower confidence limit estimate (Leung and Kupper, 1981).

Despite the relatively high odds ratio for gastroschisis associated with any recreational drug use and class A or B drugs use in the first trimester the estimated attributable risk of gastroschisis is relatively low at 6.1% (95%CI, 1.7 to 19.5) and 4.6% (95%CI, 0.9 to 20.5), respectively. This is because the prevalence of these risk factors in the control population is low (5.3% and 1.9%, respectively). Allowing for underreporting levels of two-fold in the controls and assuming a stable odds ratio the estimated attributable risk of gastroschisis associated with any recreational drug and class A or B drug use was still relatively small, 11.5% and 8.8%, respectively. Even at a three-fold level of underreporting the attributable risk of gastroschisis associated with any recreational drug and class A or B drug use was still only 15.3% and 12.6%, respectively. These findings suggest that the real population attributable risk of gastroschisis associated with recreational drug use is relatively low as underreporting levels are unlikely to be as high as three-fold.

Modifiable estimates of the proportion of gastroschisis cases attributable to other modifiable behaviours were calculated to provide a context for the interpretation of the study findings. Although the odds ratio for gastroschisis associated with cigarette smoking in the first trimester was less than two, because smoking is an extremely common exposure in this group, the estimated proportion of gastroschisis cases attributable to cigarette smoking was over one quarter. Conversely, although the odds ratio for gastroschisis associated with first trimester aspirin use was over twenty-fold the estimated proportion of gastroschisis cases attributable to aspirin use was only 8.6%, because the prevalence of use in the controls was less than one percent. The very wide 95% confidence intervals

around the attributable risk calculation for aspirin use reflect the small number of controls exposed and thus the very wide uncertainty around the estimate.

In summary, although the odds ratio for gastroschisis associated with any recreational drug use and class A or B drug use are quite high the attributable risk of these behaviours is relatively low. Therefore, if all mothers quit their recreational drug consumption the reduction in the number of gastroschisis cases would be quite small. In contrast, the attributable risk for gastroschisis associated with smoking is large and a reduction in smoking by young pregnant women would therefore have a much larger effect.

Summary and Conclusions

12.1 Summary

This study provides additional evidence to support the hypothesis that recreational drug use is a risk factor for gastroschisis (Werler et al. 2003), (Torfs et al. 1994), this finding is strengthened by the use of hair analysis to validate information collected about first trimester recreational drug use at maternal interview.

Issues of internal validity for the study have been considered. The identification of all gastroschisis cases over the study period was established using a wide network of health professionals and the three regional congenital anomalies registers. All cases were confirmed by a neonatalogist, paediatric surgeon or at pathology before inclusion within the study. Recreational drug use, the main study exposure, was validated using hair analysis in order to confirm information provided at maternal interview and to identify any underreporting of recreational drug use that may have occurred. The use of replacement controls in this study has been shown to, if anything, slightly underestimate the odds of gastroschisis associated with recreational drug use. However, every effort was made in the study design and execution to reduce these effects to a minimum.

In order to prevent any confounding by maternal age, controls were successfully matched to cases (to within one year) (Hennekens and Buring, 1987). Known putative risk factors for gastroschisis were included within the mutually adjusted unconditional logistic regression analyses. These analyses confirmed the findings that maternal exposure to vascular disruptors in the form of cigarettes and other vasoconstrictive drugs in early pregnancy increases the risk of gastroschisis (Werler et al. 2003), (Goldbaum et al. 1990), (Haddow et al. 1993), (Martinez-Frias et al. 1997), an anomaly with a suspected aetiology of vascular disruption (Hoyme et al. 1981). In addition, associations with social deprivation factors (Werler et al. 2003), (Torfs et al. 1994) and low body mass index (Lam et al. 1999) were confirmed. A possible new putative risk factor, history of gynaecological infection or disease, was identified, enhancing the description of the lifestyle of the mothers who are at risk of a gastroschisis pregnancy. However,

information about all known putative risk factors for gastroschisis was not available for the study as data were not available concerning the proximity of mothers to landfill sites (Dolk et al. 1998), (Fielder et al. 2000). If such data were made available then this environmental factor could be included within a reanalysis of the study results. As in any study, there may be unknown putative risk factors that have yet to be identified.

It is unlikely that that results from this study have been affected by chance variation as the lower limit of the 95% confidence intervals surrounding the odds ratios of 2.22 (95%CI, 1.15 to 4.26) for any recreational drug use and 3.59 (95%CI, 1.36 to 9.47) for class A or B drug use was greater than one, the value representing no difference between the cases and the controls. This study also confirms other work in this area (Werler et al. 2003), (Torfs et al. 1994). Proportionally, there were three times as many gastroschisis case mothers taking recreational drugs during the first trimester of pregnancy than there were control mothers. These odds ratios were also the highest within each model, with the exception of aspirin use, which were based on a very small number of cases. In addition, the models were found to be robust following rigorous model checking procedures.

The vascular disruption leading to a gastroschisis is thought to occur at between four and ten weeks post conception (deVries, 1980), (Hoyme et al. 1981). Data from this study confirm, both from interview and hair analysis, that vasoconstrictive drugs, in the form of any recreational drugs or class A or B drugs alone, were taken during this susceptible period of embryogenesis.

The actual dose of recreational drugs consumed was not measured in this study and therefore issues of dose-response cannot be directly addressed. However, from the four level recreational drug model it appears that there was an increasing odds ratio for gastroschisis from the use of cannabis alone and the use of class A or B drugs alone, to the use of cannabis and class A or B drugs (odds ratios 1.40, 0.89, 10.86, respectively). This tentative evidence of a gradient in risk warrants further investigation. In addition all three mutually adjusted logistic regression

models produced for the different measures of exposure contained identical putative risk factors.

Taking all these issues into consideration this study appears to have internal validity.

Consideration has also been given to issues of external validity. The source population for this study was pregnant women who were at risk of a gastroschisis pregnancy. Although it could be argued that this would be all pregnant women, because of the inverse relationship of gastroschisis with maternal age, a younger age-standardised pregnant population may be more appropriate. In this matched case control study the controls represent this population. As three-quarters of gastroschisis cases occur to mothers who are less than twenty-five years of age (Haddow et al. 1993), (Tan et al. 1996), (Werler et al. 1992a), then the study results are mainly applicable to this group of pregnant women in the UK and in westernised cultures. In addition, as previously mentioned, the findings of this study are consistent with other studies of gastroschisis (Werler et al. 2003), (Goldbaum et al. 1990), (Haddow et al. 1993), (Martinez-Frias et al. 1997) (Torfs et al. 1994) (Lam et al. 1999).

Gastroschisis is described as having a vascular pathogenesis (Hoyme et al. 1981). As such, the use of vaso-constrictive drugs during pregnancy is thought to play a causal role in gastroschisis and other anomalies thought to result from vascular disruptions (Van Allen, 1981). A number of studies have suggested that the use of recreational drugs during early pregnancy, in particular cocaine and amphetamines, which are both vaso-constrictive drugs, increases the risk of gastroschisis and other anomalies with a similar pathogenesis (Hume et al. 1997), (Torfs et al. 1994). In addition, other known vaso-constrictive agents, such as aspirin and nicotine, have also been shown to increase the risk of gastroschisis (Lam et al. 1999), (Goldbaum et al. 1990), (Werler et al. 2003). It therefore appears to be biologically plausible that recreational drug use during early pregnancy may play a causal role in the development of gastroschisis.

Taking into consideration the applicability, consistency and biological plausibility of the findings of this study it appears to have external validity.

This study was designed to test the hypothesis that use of recreational drugs in the weeks following conception is a risk factor for gastroschisis. The findings of this study confirm this hypothesis. Data collected at maternal interview indicated that mothers who used any recreational drug in the first trimester of pregnancy were over twice as likely to have a baby with a gastroschisis compared with those who did not take any recreational drugs during the first trimester. In addition, data from maternal interviews indicated that mothers who used class A or B drugs in the first trimester of pregnancy were over three and a half times more likely to have a baby with a gastroschisis compared with those who did not take class A or B drugs during the first trimester.

A unique aspect of this study was the validation of the maternal interview data using hair analysis. Although there were major problems with this analysis a minimum level of underreporting of class A or B drug use was identified. As a proportion of all identified recreational drug users 22.2% of gastroschisis case mothers and 27.3% of control mothers were identified by hair analysis. Further hair analysis may uncover higher levels of underreporting in these mothers.

The feasibility of collecting and analysing hair samples for the detection of a variety of recreational drugs and their metabolites, at a specified period of time, was tested in this study. Many problems were encountered with the hair analysis and the following conclusions were drawn. Firstly, that it is feasible to collect hair from recently pregnant mothers. Secondly, that hair analysis should be carried out in well-established laboratories to prevent errors and development problems. Thirdly, that the fineness and styling of a mother's hair should be taken into account both when collecting a sample (from the point of view of acceptability to the mother) and when selecting a small sample for timed analysis (to ensure that sufficient hair will be available for analysis). Fourthly, if the analysis of hair from a very short time window is required then the sensitivity of the hair analysis should be enhanced to ensure that any recreational drugs within the hair are detectable in samples weighing less than 5mg. Finally, hair analysis using LC-MS/MS can be

used to validate maternal interview data concerning class A or B drug use during early pregnancy.

The overall prevalence of gastroschisis in the Trent, West Midlands, and Northern health regions over the period 1st January 2001 to 31st August 2003 was amongst the highest reported rates for Europe at 4.24 per 10,000 total births (95% CI, 3.62 to 4.94). Comparative data concerning the prevalence of gastroschisis across Europe is available from the numerous congenital anomaly register members of EUROCAT (www.eurocat.ulster.ac.uk) and thus from routinely collected registry data. Consequently, there may be an element of underestimation in the reported prevalence of gastroschisis from registry data when compared to a study where there is an emphasis on ensuring the completeness of data collection. Nevertheless, even allowing for completeness of data issues the prevalence of gastroschisis in this study was very high and follows an increasing trend from Southern to Northern Europe.

12.2 Conclusions

In conclusion, this study confirms that the use of recreational drugs, in particular class A or B drugs, in the first trimester of pregnancy, is a significant risk factor for gastroschisis. The results of this study showed that mothers who used any recreational drug in the first trimester of pregnancy were over twice as likely to have a baby with a gastroschisis compared to those who did not take recreational drugs during the first trimester of pregnancy. The odds of gastroschisis was increased to over three and a half for the first trimester use of class A or B drugs.

Collection of data for the total prevalence of gastroschisis in three health regions in the UK has confirmed the strong association between young maternal age and gastroschisis with over three-quarters of the gastroschisis case mothers being under 25 years of age. The increasing prevalence of gastroschisis over time appears to be continuing, with this study recording an overall prevalence for the three health regions over the period of the study of 4.24 per 10,000 total births.

The estimated attributable risk for gastroschisis associated with reported recreational drug use, indicated that only approximately 1 in 16 gastroschisis cases were attributable to such use. For class A or B drugs, only 1 in 22 gastroschisis cases were attributable to their use. This is despite the relatively high odds ratio for gastroschisis associated with class A or B drug use in early pregnancy. Sensitivity analyses using a two to three fold level of underreporting of recreational drugs produced higher values for the attributable risk of gastroschisis associated with any recreational drug and class A or B drug use of up to 16.3% and 12.6%, respectively. The results of this study would therefore indicate that programmes developed to reduce the prevalence of gastroschisis should not concentrate on recreational drugs alone but target other modifiable risk factors, such as smoking, to have a significant effect. In addition, further work to identify as yet unknown risk factors for gastroschisis and their relationship with young mothers is required.

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Appendix A: Questionnaire

Confidential

Gastroschisis Study

Study Number | | | | | — | |

Region _____

Postcode _____

Part 1 by: _____

Interviewed by: _____

Coded by: _____

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PART 1 - Notes Review

Put a √ in the appropriate square after each question or give other details as requested.

1. Consultant _____

   Place of delivery _____

   Intended place of delivery _____

   Health District of mothers residence _____

Indicate type of Control or Case:

Healthy CONTROL ☐
Exomphalos CONTROL ☐
Gastroschisis CASE ☐

Mother's personal details:

2. Date of Birth:

Day	Month	Year

3. Marital State (tick as applicable)

Married	Single	Widowed	Separated	Divorced

If single, widowed, separated or divorced, was the mother cohabiting during pregnancy?

Yes ☐
No ☐

Family history

4. Give details of any family history of congenital anomalies:

_____
_____
_____
_____

Obstetric History

5. Using the convention (live births + stillbirths) + (abortions) write the number of such births the woman has had, including this pregnancy. (e.g. 4+2). [NB. Count births not pregnancies]

_____

Interval between previous and present pregnancy _____

6. Give details of the outcome of any previous pregnancy where a congenital anomaly was diagnosed.

_____

_____

_____

_____

Care during pregnancy

7. Initial intended place of delivery _____

8. Was antenatal care given?

Yes ☐
No ☐

If yes, what was the duration of pregnancy in weeks at the first recorded contact? _____

9. Date of first day of last menstrual period?

☐☐ ☐☐ 200☐
Day Month Year

Estimated date of delivery (by date)?

☐☐ ☐☐ 200☐
Day Month Year

Estimated date of delivery (by scan)?

☐☐ ☐☐ 200☐
Day Month Year

10. Results of fetal anomaly ultrasound scan:

Gestational age (weeks) ☐☐

Description of any anomalous findings

_____

_____

_____

11. Record any exposure to x-rays during pregnancy:

Type of x-ray _____

Date ☐☐ ☐☐ 200☐
Day Month Year

Length of pregnancy at labour and delivery

12. Gestational age at delivery (weeks) ☐☐

Type of labour

13 Indicate the type of labour: (tick one box only) ☐

- Spontaneous
- Induction – ARM
- Induction – Prostaglandins
- Augmented Labour
- Did not Labour

Mode of Delivery

14. Indicate the type of delivery the mother experienced . (tick as appropriate) ☐

- Normal (vertex or breech)
- Forceps (vertex or breech)
- Ventouse
- Breech Extraction
- Elective Caesarian
- Emergency Caesarian

If the delivery was an Caesarian section what were the indications for this method? ☐☐

_____

_____

15. **Fetal Distress**

Are any of the following mentioned in the case notes? ☐

a) Fetal distress?	No	☐	Yes	☐
b) Meconium stained liquor?	No	☐	Yes	☐
c) Type II dips?	No	☐	Yes	☐
d) Fetal bradycardia <100/min?	No	☐	Yes	☐
e) Scalp pH <7.2?	No	☐	Yes	☐

Details of Infant

16. Total number of infants born during the pregnancy under review ☐

_____

Infant's weight at birth ☐☐☐☐ grammes ☐☐☐☐

Sex of infant _____ ☐

List Apgar score readings for live births

A. 1 minute _____

B. 5 minutes _____

If stillbirth tick ☐

17. Date of Birth

		200
Day	Month	Year

Time of Birth _____hrs _____mins.
(24-hour clock)

For deaths please:

Date of Death

		200
Day	Month	Year

Time of Birth _____hrs._____mins.
(24-hour clock)

Place of Death Home

Hospital (name) _____

If hospital Ward/SCBU (specify) _____

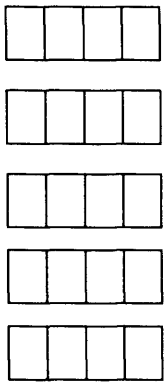
Was a post-mortem carried out?

Yes ☐
No ☐

Please photocopy post-mortem report and give cause of death:

For cases of gastroschisis/exomphalos please:

Give full details of all anomalies noted:



Please photocopy infant's hospital discharge letters

PART 2 - Maternal Interview

Put a √ in the appropriate square after each question or give other details as requested when completing the interview with the mother.

18. **Who is your G.P.?** _____

What is the address of the surgery you attend?

_____

_____Postcode_____

19. **What is your marital status?** (tick as applicable)

Married	Single	Widowed	Separated	Divorced

If not married- **did you live with your partner during pregnancy**

Yes ☐
No ☐

Occupation

If the mother was unmarried and still receiving full-time education at SCHOOL turn to Question 21. For the remainder, continue with Question 20.

20. **Did you go out to work during this pregnancy?** Yes ☐ No ☐

If 'no' turn to Question 21
If 'yes': **What was your occupation?**

_____

*If outwork please specify.
Record one occupation: if the mother had more than one job record the occupation at time of conception.

During which months of your pregnancy did you do this?

1	2	3	4	5	6	7	8	9

Month

Please tick the relevant boxes indicating the total duration of work.

In which industry did you work?

_____

Please give details of the type of work carried out.

_____

_____

Was the work full or part-time? F/T P/T

If part-time: ask number of hours worked _____

Were you self-employed? Yes ☐ No ☐

If 'yes' how many employees did you have? _____

If 'no' were you an employee? Yes ☐ No ☐

One of the following?
☐ Manager
☐ Forewoman/Supervisor
☐ Trainee
(tick one or more boxes as appropriate)

If a manager, how many employees did you care for? _____

The next questions are about your working conditions.

Did you work:

Regular days	☐	In one place	☐
Shifts on days	☐	Travelling around	☐
Shifts on nights	☐		
Regular nights	☐		
Rotating shifts	☐		
Any other	_____		

Was your workplace (tick one box from each group)

Cold	☐	Quiet	☐	Clean	☐
Warm	☐	Background Noise	☐	Dirty	☐
Hot	☐	Noisy	☐	Very Dirty	☐
Very Hot	☐	Very Noisy	☐		
Variable	☐				

Did your work involve any direct contact with:
(please specify if possible)

Solvents	_____	Yes ☐	No ☐	
Glues/adhesives	_____	Yes ☐	No ☐	
Cleaning agents	_____	Yes ☐	No ☐	
Paint spraying	_____	Yes ☐	No ☐	
Colour mixing solutions	_____	Yes ☐	No ☐	
Other chemicals	_____	Yes ☐	No ☐	

When at work did you regularly use a:
(for each specify how often)

Photocopier	_____	Yes	☐	No	☐
V.D.U.	_____	Yes	☐	No	☐
Microwave	_____	Yes	☐	No	☐
Ultrasound device	_____	Yes	☐	No	☐

For how long have you been doing this job?

(code in months) _____ ☐☐

Question 21 refers to the occupation of:

A: The father of an unmarried schoolgirl.
B: The husband of a married woman.
C: The partner of a cohabitee.

If the mother did not have a regular partner and does not fit into category **A, B, or C**, ignore question and continue with question 22.

21. What was the occupation of your father/husband/partner while you were pregnant? ☐☐☐☐

_____

In which industry did he work? ☐☐☐☐

_____

Was he self-employed? Yes ☐ No ☐

If 'yes' how many employees did he have? _____

If not self-employed was he an employee? Yes ☐ No ☐

One of the following?
☐ Manager
☐ Foreman/Supervisor
☐ Trainee

(tick one or more boxes as appropriate)

If a manager, how many employees did he care for? _____ ☐☐☐☐

22. Do any of your hobbies involve any direct contact with:
(please specify if possible)

Solvents	_____	Yes	☐	No	☐
Glues/adhesives	_____	Yes	☐	No	☐
Cleaning agents	_____	Yes	☐	No	☐
Paint spraying	_____	Yes	☐	No	☐
Colour mixing solutions	_____	Yes	☐	No	☐
Other chemicals	_____	Yes	☐	No	☐

When at home did you regularly use a:
(for each specify how often)

Photocopier	_____	Yes		No	
V.D.U.	_____	Yes		No	
Microwave	_____	Yes		No	
Ultrasound device	_____	Yes		No	

23. **What tablets, medicines, ointments or creams did you use during your pregnancy?**

For each drug ask:

i) **When did you take this?** (prompt months 1,2 or 3, the middle or end of your pregnancy)

ii) **Was this prescribed for you by a doctor?**

	Months 1	2	3	4-6	7-9
A.					
B.					
C.					
D.					
E.					
F.					
G.					
H.					
I.					
J.					

(tick one or more boxes as appropriate)

Did you take any recreational drugs during your pregnancy?
(e.g. ecstasy, crack, cannabis, LSD, amphetamines, cocaine, heroin etc.)

If yes, when did you take these? Prompt for early pregnancy use.

	Months 1	2	3	4-6	7-9
A.					
B.					
C.					
D.					
E.					
F.					
G.					
H.					
I.					
J.					

(tick one or more boxes as appropriate)

24. **In which country were you born?** ☐☐

To which ethnic group do you belong? (tick as appropriate)

- ☐ White
- ☐ Black-Caribbean
- ☐ Black-African
- ☐ Black-Other (describe)
- ☐ Indian
- ☐ Pakistani
- ☐ Bangladeshi
- ☐ Chinese
- ☐ Other ethnic group (describe) ☐

25. **What is your religion?** _____ ☐

26. **Have you ever smoked as much as one cigarette a day for as long as a year?** Yes ☐ No ☐ ☐

Between the date of your last menstrual period and your delivery did you smoke as much as one cigarette a day? Yes ☐ No ☐ ☐

Prompt: before pregnancy was confirmed.

If 'yes' during what period of the pregnancy?

1	2	3	4	5	6	7	8	9

Month ☐ ☐

On average how many cigarettes (or equivalent) per day
(One small cigar = 2 cigarettes, one large cigar = 5 cigarettes
1oz. pipe tobacco = 28 cigarettes) ☐☐

27. **Do you ever drink alcohol?** Yes ☐ No ☐ ☐

(Prompt: special occasions, homebrewed beer or wine, etc.)

Did you drink at all during this pregnancy? Yes ☐ No ☐ ☐

(Prompt: special occasions, before pregnancy was confirmed etc.)

If so, during which period of the pregnancy?

1	2	3	4	5	6	7	8	9

Month ☐ ☐

FIRST THREE MONTHS

During the first 3 months of your pregnancy, how often did you consume the following drinks?	5 or more days/ week	3-4 days/ week	1-2 days/ week	1-2 days/ month	Less than once/ month	Never	**How much of each type of drink would you consume on a typical occasion?**
Shandy							pints
Beer, lager, stout or cider							pints
Spirits or liqueurs (e.g rum, vodka, gin, whisky, advocaat, etc)							singles
Sherry or martini (including vermouth, port, conzano, etc)							glasses
Wine (include champagne & Babycham)							glasses

MONTH BEFORE ADMISSION

During the month before you came into hospital how often did you consume the following drinks?	5 or more days/ week	3-4 days/ week	1-2 days/ week	1-2 days/ month	Less than once/ month	Never	**How much of each type of drink would you consume on a typical occasion?**
Shandy							pints
Beer, lager, stout or cider							pints
Spirits or liqueurs (e.g. rum, vodka, gin, whisky, Advocaat, etc)							singles
Sherry or martini (including vermouth, port, etc)							glasses
Wine (include champagne & Babycham)							glasses

Have you been to any parties, weddings etc. where you've drunk more than usual? Yes ☐ No ☐ ☐

If so, how much did you drink, and during which month of your pregnancy did this take place?
(give details of ALL occasions) ☐☐☐

_____

28. Are your husband/partner's family and your family related in any way? Yes ☐ No ☐ ☐

29. Do you own the house you live in? Yes ☐ No ☐ ☐
(including mortgaged property)

If no:

Who do you rent your house from?
Council ☐
Landlord ☐
Housing Association ☐
Other (specify) ☐ ☐

_____

30. Do you and your family normally have the use of a car/van? Yes ☐ No ☐ ☐

31. Did you use any of the following during your pregnancy?

Hair dye ☐ ☐
Perming solution ☐ ☐
Hair bleach ☐ ☐
Other (please specify) ☐ ☐
☐

_____

HAIR SAMPLE COLLECTED: Yes ☐ No ☐ ☐

Notes:

ADDITIONAL QUESTIONS 1 – secure inside front cover

A. Mother's general medical history
Give details of past medical history including blood transfusions which had occurred before the pregnancy being investigated.
List specific disease(s):

_____
_____
_____
_____

B. Present medical history
Give details of the present medical history since the pregnancy under review (including blood transfusions, poor weight gain, problems identified before labour eg. IUGR, no fetal movements etc)

_____
_____
_____
_____

C. AFP testing
Did the mother have a serum AFP test during this pregnancy? Yes ☐ No ☐

If yes, at how many weeks gestation? _____

did it identify a problem? No ☐ Yes ☐

what was the result (in moms)? _____

Was an amniocentesis performed during this pregnancy? Yes ☐ No ☐

If yes, at how many weeks gestation? _____

what was the AFP result (in moms)? _____

Karyotype result _____

D. Maternal Height & Weight

State mother's height in feet & inches _____ or cms _____

State mother's pre-pregnancy weight in stones and pounds _____
or kilograms _____

This question is also included in the maternal interview in case the data is not available in the maternal notes.

ADDITIONAL QUESTIONS 2 – secure inside back cover

D. Maternal Height & Weight

State mother's height in feet & inches _____ or cms _____ ☐☐☐

State mother's pre-pregnancy weight in stones and pounds _____ ☐☐☐
or kilograms _____

This question is also included in the case note review.

E. Caffeine

How many cups of coffee / tea / cola did you drink (on average) per day during:

	Coffee	**Tea**	**Cola**
the first 3 months of your pregnancy ? _____	☐☐	☐☐	☐☐
months 4-6 of your pregnancy ? _____	☐☐	☐☐	☐☐
months 7-9 of your pregnancy ? _____	☐☐	☐☐	☐☐

(exclude decaffeinated drinks)

Appendix B: Prompt Cards

DRUGS PROMPT LIST: Check medicines for specific medical conditions

COUGH and DECONGESTANT PREPARATIONS:
Actifed, Benylin, Cataarh-Ex, Sudofed, Sinutab, Triominic

Prompt: Did you have a cough or cold during the first 3 months of your pregnancy? Later in pregnancy?

PAIN KILLERS: *ASPIRIN: Aspro-clear, Beecham's Powders, Lemsip, Anadin, Benylin, Alka-seltzer*
PARACETAMOL: Panadol, Migraleve, Night Nurse, Day Nurse
IBUPROFEN: Nurofen

ANTIBIOTICS: Penicillin, Amoxicillin, Flucloxacillin

VITAMINS or IRON PREPARATIONS:
Multi vitamins, Folic acid Pregaday, Ferrograd, Fefol

ANTIHISTAMINES :

ANTI-EMETICS :

STEROIDS: *Prednisolone*

CREAMS: *Hydrocortisone*

TRANQUILLISERS: *Valium, Temazepam*

DIET PILLS / APPETITE SUPPRESSANTS

RECREATIONAL DRUGS:

Acid
Trips, Tabs, Blotters, Microdots, LSD
Amphetamines
Speed, Whizz, Sulphates, Billy, Uppers
Barbiturates
Depressants, Downers, Barbs, Sleepers, Barbies
Cannabis
Marijuana, Grass, Dope, Draw, Puff, Blow, Weed, Gear, Spliff, Ganja, Herb, Wacky Backy, Green, Bud, Skunk, Resin, Hash, Pot, Dope, Shit, Black, Gold, Brown, Slate, Squidgy
Cocaine
Coke, Charlie, Toot, Chang, Snow, White Lady, Bolivian Marching Powder
Crack
Rock, Wash, Stone, Roxanne, Cloud, Flake, Nuggets, Nine, Base, Baseball
DMT
The Businessman's Trip, The Businessman's Lunch
Ecstasy
E, Love Doves, Clarity, Adam, Disco Biscuits, Shamrocks, MDMA, X, XTC
GHB (gammahydroxybutyrate)
GBH (Grievous Bodily Harm), Liquid Ecstasy, Liquid X
Herbal Highs
Herbal Ecstasy, Herbal X-Tacy, Cloud 9, Khat, Qat, Quat
Heroin
H, Smack, Junk, Horse, Harry, Brown, Gravy
Ketamine
K, Special K, Vitamin K
Magic Mushrooms
Shrooms, Mushies
Methadone
Dolly, Doll, Red Rock, Phy-Amps, PHY
Methylamphetamine (or methamphetamine)
Ice, Crystal, Meth, Ice Cream, Glass
PCP
Angel Dust, Elephant Tranquilliser, Rocket Fuel, Zombie, Whack, Embalming Fluid
Poppers
Amyl, Rush, Rave, Stage, Liquid Gold, Stud, Ram
Solvents
Glue, Gas, Huff, Aerosols
Tranquillizers
Tranx, Benzos, Blockers, Blockbusters, Chewies, Jellies, Eggs, Rugby Balls, Temazzies, M&Ms

RECREATIONAL DRUGS:

Cannabis
Ecstasy
Magic Mushrooms
Herbal Highs
Tranquillizers
PCP
Poppers Amphetamines
Barbiturates
DMT
GHB (gammahydroxybutyrate)
Methadone
Methylamphetamine (or methamphetamine)
Solvents
Cocaine
Crack
Acid
Heroin

Appendix C: Letter to Clinicians and Study Abstract

Our Ref: ESD/jmd/TIMMS/gastro/lets/TrentCons.doc
31st January 2001

To: All
Consultant Obstetricians/Paediatricians
Neonatologists
Perinatal Pathologists
Paediatric Surgeons
Superintendent Ultrasonographers
in Trent Region

Dear Colleague,

Recreational Drug Use: a major risk factor for gastroschisis.

We are writing to you to ask for your support for the above project. An abstract of this study is enclosed with this letter. In summary we plan to set up a study to measure the risk associated with the use of recreational drugs during early pregnancy and the incidence of gastroschisis. This study will involve the collaboration of three congenital anomalies registers: Trent, West Midlands and Northern Regions which will identify the cases of gastroschisis and exomphalos. This study is being funded by the Regional R&D Programmes for Trent, West Midlands and Northern regions. This study has Trent MREC approval and also approval from all relevant LRECs.

We hope to include all mothers of infants with gastroschisis or exomphalos plus a sample of mothers with healthy infants, who will be identified from labour ward registers. **Please would you discuss this study with all women who either terminate a fetus or deliver an infant with either of these conditions** to prepare for these mothers to be approached either personally by a research midwife/health visitor on the maternity or neonatal unit, or by letter, following discharge home. Copies of the information sheets and letters to be used are enclosed.

It has been shown that the co-operation of women, in similar studies, is enhanced when the initial letter of approach is sent from their hospital (on appropriately headed paper as specified by ethical committees). In the case of obstetric consultants, we would like to obtain your permission to approach your patients.

If you have any questions or problems concerning this study please contact me at the address below or on 0116 252 3200

Thanking you in anticipation

Yours sincerely

Elizabeth S Draper
Deputy Director TIMMS /
Trent Congenital Anomalies Register

Abstract of Research

The birth incidence of gastroschisis has shown a two to threefold increase, worldwide, over the past two decades, which appears to be concentrated in births from teenage pregnancies. Current aetiological theories suggest that gastroschisis results from a disruption or compromise of the right omphalomesenteric artery, suggesting environmental or endogenous maternal risk factors.

In recent years there has been a dramatic increase in the prevalence of recreational drug use, particularly in those aged 16 to 19 years. The prevalence of recreational drug use during pregnancy and the subsequent impact upon the fetuses of pregnant women, within this group is, however, unknown. Research has indicated increased morbidity within this group, with an increased prevalence of growth retarded infants and a possible increase in the incidence of gastroschisis.

This project will:

(i) test the hypothesis that the incidence of gastroschisis is positively associated with the use of recreational drugs in the weeks following conception;

(ii) test the secondary hypothesis that there is no association between the incidence of exomphalos and the use of recreational drugs in the weeks following conception.

A case-control study is proposed to investigate these hypotheses. Case note review and maternal interviews will be used to collect information about risk factors for gastroschisis. Human hair will be collected for analysis to validate interview data concerning recreational drug use. Antenatal ascertainment of gastroschisis cases will be provided by three Regional Congenital Anomalies Registers: Trent, West Midlands and Northern. For each case, three live birth controls will be selected, matched by district and maternal age (to within one year).

Appendix D: Information Sheets

Hospital Headed Notepaper - (Surviving Cases)

Information Sheet for the Infant Abdominal Wall Defect Study

Thankyou for reading this information sheet.

We are sorry to hear about your baby's problem. You are invited to take part in a research study, but before you agree to take part it is important for you to understand what it will involve. Please read the following information carefully and discuss it with your friends and family if you wish.

What is the purpose of this study? This study is trying to find out why abdominal wall defects occur and in particular if there is any link between substances that women may have been exposed to during pregnancy and their baby having an abdominal wall defect.

Why have I been chosen? All mothers of babies born with an abdominal wall defect are being asked to take part in this study.

Who is organising the study? The study is being organised by the Trent Congenital Anomalies Register (West Midlands/ Northern as applicable) and is being funded by the *NHS Policy & Practice Research and Development Programme.*

What will happen to me if I take part? The study involves you agreeing to a brief interview and allowing us to take a few strands of your hair for analysis. We are going to ask you a number of questions including details of your use of any type of drug during your pregnancy (prescribed or any others, ie. from aspirin to recreational drugs). We will also ask if you will allow us to collect a small, unnoticeable sample of hair from the back of your head which will be tested in line with the questionnaire. The whole thing takes less than 15 minutes.

Do I have to take part? Your taking part in this study is voluntary and will not affect either the treatment of yourself or your baby.

Who will see my records and know about my taking part? All information collected for this study is **completely confidential.** Any information about you which leaves the hospital will be anonymous so that you cannot be recognised from it.

We hope that you will agree to take part in this project as it may help us to improve the health of pregnant women and their babies.

Thankyou for your help.

If you have any problems, concerns, complaints or other questions about this study please contact: Elizabeth S Draper at the Department of Epidemiology & Public Health, 22-28, Princess Road West, Leicester LE1 6TP or telephone 0116 2523200. Alternatively you may contact the Complaints Department *on telephone ..... @ appropriate health authority or hospital*

Version 1.7 13/04/99

Hospital Headed Notepaper - (Cases: TOPs & Deaths)

Information Sheet for the Infant Abdominal Wall Defect Study

Thankyou for reading this information sheet.

We are sorry to hear about the loss of your baby. As you know, your baby had a serious defect of the abdominal wall. We are trying to find out more about this problem and you are invited to take part in a research study, but before you agree to take part it is important for you to understand what it will involve. Please read the following information carefully and discuss it with your friends and family if you wish.

What is the purpose of this study? This study is trying to find out why abdominal wall defects occur and in particular if there is any link between substances that women may have been exposed to during pregnancy and their baby having an abdominal wall defect.

Why have I been chosen? All mothers of babies born with an abdominal wall defect are being asked to take part in this study.

Who is organising the study? The study is being organised by the Trent Congenital Anomalies Register (West Midlands/ Northern as applicable) and is being funded by the *NHS Policy & Practice Research and Development Programme.*

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Do I have to take part? Your taking part in this study is voluntary and will not affect your treatment.

Who will see my records and know about my taking part? All information collected for this study is **completely confidential.** Any information about you which leaves the hospital will be anonymous so that you cannot be recognised from it.

We hope that you will agree to take part in this project as it may help us to improve the health of pregnant women and their babies.

Thankyou for your help.

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Version 1.7 13/04/99

Hospital Headed Notepaper - (Surviving Cases)

Information Sheet for the Infant Abdominal Wall Defect Study
For parental consent cases

Thankyou for reading this information sheet.

We are sorry to hear about your grandchild's problem. Your daughter is being invited to take part in a research study, but before you consent for her to take part it is important for you to understand what it will involve. Please read the following information carefully and discuss it with your friends and family if you wish.

What is the purpose of this study? This study is trying to find out why abdominal wall defects occur and in particular if there is any link between substances that women may have been exposed to during pregnancy and their baby having an abdominal wall defect.

Why has my daughter been chosen? All mothers of babies born with an abdominal wall defect are being asked to take part in this study.

Who is organising the study? The study is being organised by the Trent Congenital Anomalies Register (West Midlands/ Northern as applicable) and is being funded by the *NHS Policy & Practice Research and Development Programme*.

What will happen to my daughter if she takes part? The study involves a brief interview with your daughter and your consenting to allow us to take a few strands of her hair for analysis. We are going to ask a number of questions including details of her use of any type of drug during her pregnancy (prescribed or any others, ie. from aspirin to recreational drugs). We will also ask if you will allow us to approach you daughter to collect a small, unnoticeable sample of hair from the back of her head which will be tested in line with the questionnaire. The whole thing takes less than 15 minutes.

Does my daughter have to take part? Taking part in this study is voluntary and will not affect either the treatment of your daughter or her baby.

Who will see my daughters records and know about her taking part? All information collected for this study is **completely confidential.** Any information about your daughter which leaves the hospital will be anonymous so that she cannot be recognised from it.

We hope that you will consent for your daughter to take part in this project as it may help us to improve the health of pregnant women and their babies.

Thankyou for your help.

If you have any problems, concerns, complaints or other questions about this study please contact: Elizabeth S Draper at the Department of Epidemiology & Public Health, 22-28, Princess Road West, Leicester LE1 6TP or telephone 0116 2523200. Alternatively you may contact the Complaints Department *on telephone ..... @ appropriate health authority or hospital*

Version 1.7 13/04/99

Hospital Headed Notepaper - (Cases: TOPs & Deaths)

Information Sheet for the Infant Abdominal Wall Defect Study
For parental consent cases

Thankyou for reading this information sheet.

We are sorry to hear about the loss of your grandchild. As you know, your daughter's baby had a serious defect of the abdominal wall. We are trying to find out more about this problem and your daughter is being invited to take part in a research study, but before you consent for her to take part it is important for you to understand what it will involve. Please read the following information carefully and discuss it with your friends and family if you wish.

What is the purpose of this study? This study is trying to find out why abdominal wall defects occur and in particular if there is any link between substances that women may have been exposed to during pregnancy and their baby having an abdominal wall defect.

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back of her head which will be tested in line with the questionnaire. The whole thing takes less than 15 minutes.

Does my daughter have to take part? Taking part in this study is voluntary and will not affect either the treatment of your daughter.

Who will see my daughters records and know about her taking part? All information collected for this study is **completely confidential.** Any information about your daughter which leaves the hospital will be anonymous so that she cannot be recognised from it.

We hope that you will consent for your daughter to take part in this project as it may help us to improve the health of pregnant women and their babies.

Thankyou for your help.

If you have any problems, concerns, complaints or other questions about this study please contact: Elizabeth S Draper at the Department of Epidemiology & Public Health, 22-28, Princess Road West, Leicester LE1 6TP or telephone 0116 2523200. Alternatively you may contact the Complaints Department *on telephone ..... @ appropriate health authority or hospital*

Version 1.7 13/04/99

Hospital Headed Notepaper - (Controls)

Information Sheet for the Infant Abdominal Wall Defect Study

Thankyou for reading this information sheet.

You are invited to take part in a research study, but before you agree to take part it is important for you to understand what it will involve. Please read the following information carefully and discuss it with your friends and family if you wish.

What is the purpose of this study? This study is trying to find out why some babies are born with a defect where the intestines protrude through a hole in the abdomen, in particular, if there is any link between substances that women may have been exposed to during pregnancy and this defect.

Why have I been chosen? You have been selected as part of a sample of women who have recently delivered a healthy baby - **a comparison group.**

Who is organising the study? The study is being organised by the Trent Congenital Anomalies Register (West Midlands/ Northern as applicable) and is being funded by the *NHS Policy & Practice Research and Development Programme.*

What will happen to me if I take part? The study involves you agreeing to a brief interview and allowing us to take a few strands of your hair for analysis. We are going to ask you a number of questions including details of your use of any type of drug during your pregnancy (prescribed or any others, ie. from aspirin to recreational drugs). We will also ask if you will allow us to collect a small, unnoticeable sample of hair from the back of your head which will be tested in line with the questionnaire. The whole thing takes less than 15 minutes.

Do I have to take part? Your taking part in this study is voluntary and will not affect either the treatment of yourself or your baby.

Who will see my records and know about my taking part? All information collected for this study is **completely confidential.** Any information about you which leaves the hospital will be anonymous so that you cannot be recognised from it.

We hope that you will agree to take part in this project as it may help us to improve the health of pregnant women and their babies.

Thankyou for your help.

If you have any problems, concerns, complaints or other questions about this study please contact: Elizabeth S Draper at the Department of Epidemiology & Public Health, 22-28, Princess Road West, Leicester LE1 6TP or telephone 0116 2523200. Alternatively you may contact the Complaints Department *on telephone ..... @ appropriate health authority or hospital*

Version 1.7 13/04/99

Hospital Headed Notepaper - (Controls)

Information Sheet for the Infant Abdominal Wall Defect Study
For parental consent cases

Thankyou for reading this information sheet.

Your daughter is being invited to take part in a research study, but before you consent for her to take part it is important for you to understand what it will involve. Please read the following information carefully and discuss it with your friends and family if you wish.

What is the purpose of this study? This study is trying to find out why some babies are born with a defect where the intestines protrude through a hole in the abdomen, in particular, if there is any link between substances that women may have been exposed to during pregnancy and this defect.

Why has my daughter been chosen? Your daughter has been selected as part of a sample of women who have recently delivered a healthy baby - **a comparison group.**

Who is organising the study? The study is being organised by the Trent Congenital Anomalies Register (West Midlands/ Northern as applicable) and is being funded by the *NHS Policy & Practice Research and Development Programme.*

What will happen to my daughter if she takes part? The study involves a brief interview with your daughter and your consenting to allow us to take a few strands of her hair for analysis. We are going to ask a number of questions including details of her use of any type of drug during her pregnancy (prescribed or any others, ie. from aspirin to recreational drugs). We will also ask if you will allow us to approach you daughter to collect a small, unnoticeable sample of hair from the

back of her head which will be tested in line with the questionnaire. The whole thing takes less than 15 minutes.

Does my daughter have to take part? Taking part in this study is voluntary and will not affect either the treatment of daughter or her baby.

Who will see my daughters records and know about her taking part? All information collected for this study is **completely confidential.** Any information about your daughter which leaves the hospital will be anonymous so that she cannot be recognised from it.

We hope that you will consent for your daughter to take part in this project as it may help us to improve the health of pregnant women and their babies.

Thankyou for your help.

If you have any problems, concerns, complaints or other questions about this study please contact: Elizabeth S Draper at the Department of Epidemiology & Public Health, 22-28, Princess Road West, Leicester LE1 6TP or telephone 0116 2523200. Alternatively you may contact the Complaints Department *on telephone ..... @ appropriate health authority or hospital*

Version 1.7 13/04/99

Appendix E: Consent Forms

TRENT ABDOMINAL WALL DEFECT STUDY

Agreement Form
To be completed by the subject

Please circle correct response.

Have you read the information sheet? (Please take a copy home with you to keep)	YES / NO
Have you had an opportunity to ask questions and to discuss the study?	YES / NO
Have you had satisfactory answers to all of your questions?	YES / NO
Do you give permission for the research midwife/ health visitor to have access to your medical records?	YES / NO
Do you agree to a small sample of your hair being taken?	YES / NO
Do you agree to take part in the study?	YES / NO

Signature_____ Date_____

Name in block letters _____

I have explained the study to the above subject and she has indicated her willingness to take part.

Research Midwife/HV signature_____

Version 1.7 13/04/99

TRENT ABDOMINAL WALL DEFECT STUDY

Agreement Form
To be completed by the parent/guardian for under age subjects.

Please circle correct response.

Have you read the information sheet? YES / NO
(Please take a copy home with you to keep)

Have you had an opportunity to ask questions and to discuss the study? YES / NO

Have you had satisfactory answers to all of your questions? YES / NO

Do you give permission for the research midwife to have access to your daughters medical records? YES / NO

Do you agree to a small sample of your daughters hair being taken? YES / NO

Do you consent to your daughter taking part in the study? YES / NO

Signature_____ Date_____

(Parent/guardian)

Name in block letters _____

I have explained the study to the above subject and she has indicated her willingness for her daughter to take part.

Research Midwife/HV signature_____

Version 1.7 13/04/99

Appendix F: Coding Manual

Gastroschisis Study

CODING INSTRUCTIONS

`Not applicable' - code as 8 all boxes (N.A)

`Nil apparent' - code as 0 all boxes

Data `not available' - code as 9 all boxes (N.K)

'Normal result' – code as 777

COVER

Survey number - to be written as an alpha-numeric (see instructions for Question 1)

Ensure that details of the mother's postcode and region are completed on the front of the questionnaire. (Postcode for 'Out of Country' is ZZ99 9ZZ).

Please complete details of who completed:

Part 1 _____

Interview _____

Coding _____

Q No.	Variable name	Subject	Coding instructions	Code
1.		Consultant	Do not code	
	POD	Place of delivery	**TRENT**	
			Barnsley	1
			Rotherham	2
			Doncaster	3
			Jessop	4
			Northern General	5
			Chesterfield	6
			Bassetlaw	7
			Kings Mill	8
			Derby City	9
			Nottingham City	10
			Queens Medical Centre	11
			Lincoln	12
			Pilgrim	13
			Leicester RI	14
			Leicester GH	15
			Grimsby	16
			Scunthorpe	17
			Grantham	18
			St Mary's Melton	19
			WEST MIDLANDS	
			Alexandra Hospital	20
			Birmingham Women's Hospital	21
			City Hospital	22
			George Eliot Hospital	23
			Good Hope Hospital	24
			Birmingham Heartlands Hospital	25
			County Hospital, Hereford	26
			Kidderminster General Hospital	27
			New Cross Hospital,	28
			Wolverhampton	29
			North Staffs Maternity Hospital	30
			Queens Hospital, BoT	31
			Royal Shrewsbury Hospital	32
			Sandwell District General Hospital	33
			Solihull Hospital	34
			Stafford District Hospital	35
			Manor Hospital, Walsall	36
			Walsgrave Hospital	37
			Warwick Hospital	38
			Worcester RI (Ronkswood)	39
			Wordsley Hospital	40
			NORTHERN	
			University Hospital Nth Durham	50
			Hartlepool General	51
			Cameron Hospital	52
			North Tees General	53
			Carter Bequest	54
			South Cleveland	55
			Guisbrough	56
			Middlesborough	57

		Cumberland Infirmary (Carlisle)	58
		City Maternity	59
		Penrith	60
		Ruth Lancaster	61
		West Cumberland	62
		Darlington Memorial	63
		Dryburn	64
		Shotley Bridge	65
		Bishop Aukland	66
		Sunderland	67
		Queen Elizabeth (Gateshead)	68
		South Tyneside	69
		RVI	70
		Princess Mary	71
		Newcastle General	72
		Freeman	73
		North Tyneside	74
		Preston	75
		Berwick Infirmary	76
		Alnwick Infirmary	77
		Ashington	78
		Hexham	79
		Out of Region	80
		HOME	90
		Northallerton	81
		Private Clinic (Anywhere)	85
IPOD	Intended place of delivery at onset of labour	Code as previous list	
HD	Health District	**TRENT**	
		North Derbys	1
		South Derbys	2
		Leicestershire	3
		Lincolnshire	4
		North Notts	5
		Nottingham	6
		Barnsley	7
		Doncaster	8
		Rotherham	9
		Sheffield	10
		South Humber	11
		WEST MIDLANDS	
		Coventry	20
		Dudley	21
		Herefordshire	22
		North Staffordshire	23
		Sandwell	24
		Shropshire	25
		Solihull	26
		South Staffordshire	27
		Walsall	28
		Warwickshire	28
		Wolverhampton	30
		Worcestershire	31

			Birmingham	32
			NORTHERN	
			Tees	40
			North Cumbria	41
			Durham	42
			Sunderland	43
			Gateshead & South Tyneside	44
			Newcastle & North Tyneside	45
			Northumberland	46
	CSCN	Type of case or control	Healthy control	1
			Exomphalos control	2
			Gastroschisis case	3
2.	MOTHDAY MOTHMON MOTHYR	Maternal DOB	Code as date of birth FORMAT	 ddmmyyyy
3.	MS	Marital state	Married	1
			Single	2
			Widowed	3
			Separated	4
			Divorced	5
	COHAB	If single, widowed, separated or divorced, co-habiting whilst pregnant?	If not ticked check with Q.19	
			No	0
			Yes	1
			Married women living with husband	8
4.	FHIST1 FHIST2	Family history of conmals?	Code to ICD10 Chapter XVII eg. Gastroschisis Q79.3, Omphalocele Q72.2	
			Nil apparent code	0000
5.	PAR1 PAR2	Indication of parity	Code 2+2 as 22 ie. 2 live or SB & 2 abortions Code 9 or more as 9 eg. 10+2 code as 92 In the event of aborted twins include each twin eg. if patient has previously aborted twins and subsequently has a stillbirth code 1+2 as 12	
	YSLP	Years since last pregnancy	Interval in years since last pregnancy – code in years	
			eg. less than 1 year code	01
			1-2 years	02
			2-3 years	03
			Over 4 years	04
			not applicable	88

6.	PREVAB1 PREVAB2	Details of previous pregnancies complicated by an anomaly	Code to ICD10 Chapter XVII eg. Gastroschisis Q79.3 Nil apparent code	 0000
7.	IIPOD	Initial intended place of delivery	Code as list in question 1.	
8.	ANC	Was antenatal care given?	Yes No	1 0
	ANCGEST	Gestation at first recorded antenatal visit	Code as completed weeks gestation	
9.		LMP dates	Not coded	
10.	USGEST	Gestation at fetal anomaly scan	Code as completed weeks gestation If no fetal anomaly scan carried out – code as n/a	 88
	ANOM1 ANOM2 ANOM3	Anomalous findings	Code to ICD10 Chapter XVII eg. Gastroschisis Q79.3 Nil apparent code	 0000
11.	XRAY	Type of X-ray	Ordinary CAT Scan MRI Radio-isotope studies Other None	1 2 3 4 5 0
	DAYXRAY MONXRAY YRXRAY	Date of X-ray	Code as date of procedure FORMAT **Code date nearest to first trimester**	 ddmmyyyy
12.	GEST	Duration of pregnancy at delivery	Code as completed weeks CHECK WITH CALENDAR	
13.	TYPELAB	Type of labour	Spontaneous Induction – ARM Induction – prostaglandins etc Augmented labour Induction – ARM + prostin Other Induction Induction + Augmented Labour Unknown Did not labour	1 2 3 4 5 6 7 8 0
14.	DEL	Mode of delivery	Normal (vertex or breech) Forceps (vertex or breech) Ventouse Breech Extraction Elective Caesarian Emergency Caesarian	1 2 3 4 5 6

	INDCS	Reason for caesarian?	Foetal distress	1
			Maternal PET / raised BP	2
			Abnormal pelvimetry	3
			Unstable /abnormal lie	4
			Previous section	5
			Multiple pregnancy	6
			Placenta praevia	7
			Prolapsed cord	8
			Ruptured uterus	9
			Placental insuffieciency	10
			Delayed /prolonged labour	11
			Concealed abruption	12
			Breech	13
			Failure to progress	14
			Other	15
15.	FDIST	Fetal distress	Yes	1
			No	0
	MECON	Meconium stained liquor	Yes	1
			No	0
	DIPS	Type II dips	Yes	1
			No	0
	BRADY	Fetal bradycardia <100	Yes	1
			No	0
	PH	Scalp pH<7.2	Yes	1
			No	0
16.	MULT	Number of infants born during the pregnancy under review	Code as number of infants	
	BWT	Infant's weight at birth	Code as weight in grammes	
	SEX	Sex of infant	Male	1
			Female	2
			Indeterminate	3
	APGAR1 APGAR5	Apgar score readings	Code as scores at 1 and 5 minutes	
			Stillbirth code boxes	00
			Not known code boxes	99
17.	DAY MONTH YEAR	Date of infant's birth	Code as date FORMAT	ddmmyyyy
	HOURS MINUTES	Time of infant's birth	Code as time using 24 hour clock	
18.	DDEATH MDEATH YDEATH	Date of infants death if applicable	Code as date FORMAT	ddmmyyyy
			N/A	88888888

	HRDEATH MNDEATH	Time of infants DEATH	Code as time using 24 hour clock	
	HOME	Place of death	Home	01
	HOSPITAL	Place of death	Code as list in question 1	
	WARD	If hospital ?ward	Maternity ward NNU/SCBU PICU General paediatric ward Other	1 2 3 4 5
	PM	Post mortem	Yes No	1 0
	COD1 COD2 COD3	Cause of death from post mortem	Code to ICD10 Chapter XVII eg. Gastroschisis Q79.3 Nil apparent code	 0000
	PMANOM1 PMANOM2 PMANOM3 PMANOM4 PMANOM5	Anomalies	Code to ICD10 Chapter XVII eg. Gastroschisis Q79.3 Nil apparent code	 0000
18.		Not coded		
19.	MSMUM	Marital state	Married Single Widowed Separated Divorced	1 2 3 4 5
	COHABMUM	If single, widowed, separated or divorced, co-habiting whilst pregnant?	No Yes Married women living with husband	0 1 8
20.	WORK	Did mother work during pregnancy?	Yes No	1 0
	OCCUP	If yes, state occupation	Code as Standard Occupational Classification Student code Housewife / unemployed (CODE IN LEICESTER)	 7777 8888
	ENDWK	Period of work	Code as number of months pregnant when finished working	
	IND	Industry	Code as Standard Industrial classification manual. Housewife/student/unemployed (CODE IN LEICESTER)	 8888

FTPT	Full or part-time work	Full time	1
		Part time	2
HOURSW	No of hours worked for part time	Code as hours (per week)	
SCLASS	Social class (1st 2 of the 4 boxes)	Code as Standard Occupational Classification manual (CODE IN LEICESTER)	
		Social class I	1n
		Social class II	2n
		Social class IIInM	3n
		Social class IIIM	3m
		Social class IV	4m
		Social class V	5m
		Forces	0
		Student	7
		Housewife /unemployed	8
SEG	Socio-economic group (boxes 3&4)	Code as Standard Occupational Classification manual (CODE IN LEICESTER)	
		Students	77
		Housewife / unemployed	17
		Forces	16
SHIFT	Pattern of work	Regular days	1
		Shifts on days	2
		Shifts on nights	3
		Regular nights	4
		Rotating shifts	5
		Other	6
AROUND	Where worked	In one place	1
		Travelling around	2
		Both	3
		Other	4
TEMP	Conditions	Cold	1
		Warm	2
		Hot	3
		Very Hot	4
		Variable	5
NOISE	Conditions	Quiet	1
		Background noise	2
		Noisy	3
		Very Noisy	4
		Variable	5
CLEAN	Conditions	Clean	1
		Dirty	2
		Very dirty	3
		Variable	4

	SOLVENT GLUE AGENT PAINT COLOUR OTHER	Use of chemicals at work	Code all: Yes No	 1 0
	COPIER VDU MICRO USOUND	Use of office machinery at work	Code all: Yes No	 1 0
	JOBMTHS	How long in present employment	Code in months	
21	FOCCUP	Occupation of husband, cohabiting partner or father of a schoolgirl	Code as Standard Occupational Classification Student code Unemployed (CODE IN LEICESTER)	 7777 8888
	FIND	Industry	Code as Standard Industrial classification manual. Student/unemployed (CODE IN LEICESTER)	 8888
	FSCLASS	Social class (1st 2 boxes of 4)	Code as Standard Occupational Classification manual (CODE IN LEICESTER) Social class I Social class II Social class IIInM Social class IIIM Social class IV Social class V Forces Student Unemployed	 1n 2n 3n 3m 4m 5m 0 7 8
	FSEG	Socio-economic group (boxes 3&4)	Code as Standard Occupational Classification manual (CODE IN LEICESTER) Students Housewife / unemployed Forces	 77 17 16
22	HSOLVENT HGLUE HAGENT HPAINT HCOLOUR HOTHER	Use of chemicals for a hobby	Code all Yes No	 1 0

	HCOPIER HVDU HMICRO HUSOUND	Use of office machinery at home	Yes No	1 0
23	DRUGA DRUGB DRUGC DRUGD DRUGE DRUGF DRUGG DRUGH DRUGI DRUGJ	Tablets, medicines, ointments, creams used by mother during pregnancy. Code 1st 5 of the 7 boxes	Code as BNF section headings Include all drugs said to have been taken List of drugs etc commonly used on separate sheet	
	WHENA...to WHENJ	Indicate months when taken Box 6 of the 7	Month 1 Month 2 Month 3 Months 4-6 Months 7-9 1st & 2nd trimester 1st & 3rd trimester 2nd & 3rd trimester All trimesters Months 1 & 2 Months 2 & 3 Months 1 & 3 Months 1 & 2 & 3 Months 2 & 3 & 2nd trimester Month 3 & 2nd trimester Month 1 & 2nd trimester Months 3 & 2nd+3rd trimester Months 1 & 3 + 3rd trimester Months 1 & 2 + 3rd trimester Months 2 & 3 + 2nd + 3rd trimester Months 2 + 2nd trimester Month 1 + 2nd+3rd trimester Months 1+3 + 2nd+3rd trimester Months 2+3 + 3rd trimester Months 2 + 2nd+3rd trimester Month 2 + 3rd trimester Month 1 + 3rd trimester Months 3 + 3rd trimester	1 2 3 4 5 6 7 8 A B C D E F G H I J K L M N P Q R S T U
	PRESCA...to PRESCJ	Indicate if prescribed by a doctor Box 7 of the 7	Yes No	1 0
	RECDA RECDB ...to RECDJ	Recreational drugs used by mother during pregnancy	Code using attached list	
	TRIMA...to TRIMJ	Indicate months when taken Box 6 of the 7	Month 1 Month 2 Month 3 Months 4-6	1 2 3 4

			Months 7-9	5
			1st & 2nd trimester	6
			1st & 3rd trimester	7
			2nd & 3rd trimester	8
			All trimesters	A
			Months 1 & 2	B
			Months 2 & 3	C
			Months 1 & 3	D
			Months 1 & 2 & 3	E
			Months 2 & 3 & 2nd trimester	F
			Month 3 & 2nd trimester	G
			Month 1 & 2nd trimester	H
			Months 3 & 2nd+3rd trimester	I
			Months 1 & 3 + 3rd trimester	J
			Months 1 & 2 + 3rd trimester	K
			Months 2 & 3 + 2nd + 3rd trimester	L
			Months 2 + 2nd trimester	M
			Month 1 + 2nd+3rd trimester	N
			Months 1+3 + 2nd+3rd trimester	P
			Months 2+3 + 3rd trimester	Q
			Months 2 + 2nd+3rd trimester	R
			Month 2 + 3rd trimester	S
			Month 1 + 3rd trimester	T
			Months 3 + 3rd trimester	U
24	COB	Mother's country of birth	United Kingdom	1
			Irish republic	2
			Old commonwealth ie. Australia Canada New Zealand	3
			Africa	4
			America USA	5
			Caribbean	6
			India	7
			Pakistan	8
			Other countries in Asia / Oceania	9
			Europe	10
			Other	11
			Unknown	99
	ETH	Mother's ethnic group	White	1
			Black – Caribbean	2
			Black - African	3
			Black - Other	4
			Indian	5
			Pakistani	6
			Bangladeshi	7
			Chinese	8
			Other ethnic group	0
			Unknown	9
25	REL	Mother's religion	Hindu	1
			Moslem	2
			Sikh	3
			Christian	4
			Other	5
			None	0

26	EVERSK	Has mother ever smoked one cigarette a day for as long as a year?	No Yes	0 1
	SMOKE	Between LMP and delivery - smoked one or more cigarettes per day	No Yes	0 1
	FSMOK	If 'yes' during what period of pregnancy	Month of pregnancy smoking began - code as month eg. 1st=1	
	TOTSMO	Total months of pregnancy during which the mother smoked	Code as total months This box is missing on the questionnaire – please write number next to FSMOK box.	
	CIGS	How many cigarettes per day	Code as number	
27	EVDRINK	Drink alcohol ever?	No Yes	0 1
	DRINK	Did mother drink alcohol during pregnancy?	No Yes	0 1
	FDRINK TOTDRINK	During what period of pregnancy	Code as for smoking Qs	

ALCOHOL CONSUMED DURING FIRST 3 MONTHS OF PREGNANCY - code all empty boxes 0

FREQ1	Frequency of drinking shandy	5 or more days per week 3-4 days per week 1-2 days per week 1-2 days per month Less then once per month Never	5 4 3 2 1 0
AMT1	Number of units of shandy consumed on a typical occasion	1 pint shandy = 1 unit	
FREQ2	Frequency of drinking beer etc	Code as for shandy	
AMT2	Number of units of beer consumed on a typical occasion	Half pint beer = 1 unit	
FREQ3	Frequency of drinking spirits	Code as for shandy	
AMT3	Number of units of spirits consumed	1 single measure = 1 unit 25 ml in a measure	

	on a typical occasion	75 cl in a bottle 30 measures in a bottle
FREQ4	Frequency of drinking sherry etc	Code as for shandy

NB: ALCOPOPS = 1.7 (ROUND UP TO NEAREST AMOUNT)

	AMT4	Number of units of sherry consumed on a typical occasion	1 glass (2 fl oz) = 1 unit 75 cl in a bottle (20 floz) = 10 units	
	FREQ5	Frequency of drinking wine etc	Code as for shandy	
	AMT5	Number of units not wine consumed on a typical occasion	1 glass (4-4.5 fl oz) = 1 unit	

ALCOHOL CONSUMED DURING MONTH PRIOR TO HOSPITAL ADMISSION - code all empty boxes 0

	FREQ6 AMT6	Shandy	Code as for FREQ1 and AMT1	
	FREQ7 AMT7	Beer etc	Code as for FREQ2 and AMT2	
	FREQ8 AMT8	Spirits	Code as for FREQ3 and AMT3	
	FREQ9 AMT9	Sherry	Code as for FREQ4 and AMT4	
	FREQ10 AMT10	Wine	Code as for FREQ5 and AMT5	
	BINGE	More alcohol consumed than usual?	Yes No	1 0
	BGMTH	If yes month of binge	Code as number of months pregnant	
	BGAMT	Amount consumed	Code in units of alcohol SEE AMT1 to AMT5	
28	CONSANG	Blood relation with partner?	Yes No	1 0
29	OWNER	House owner?	Yes No	1 0
	RENT	If no, who is house rented from?	Council Landlord Housing Association Other (specify) Non rented property (eg lives with parents)	1 2 3 4 0
30	CAR	Use of car	Yes No	1 0
31	DYE	Use of hair	Code as :	

	PERM BLEACH OTHAIR	applications	Yes No For each	1 0
	HSAMPLE	Hair sample collected	Yes No	1 0

ADDITIONAL QUESTIONS AT FRONT & BACK OF QUESTIONNAIRE

A.	PASTH1 PASTH2 PASTH3	Mother's past medical history	Code as ICD10 manual - sheet of frequently used codes attached	
B.	PRESH1 PRESH2 PRESH3	Mother's current medical & obstetric history	Code as ICD10 manual - sheet of frequently used codes attached	
C.	AFP	Did mother have a serum AFP test?	Yes No	1 0
	AFPGEST	How many weeks gestation at test?	Code as number of completed weeks	
	PROBLEM	Did it identify a problem?	Yes No	1 0
	RESULT	What was the result?	Code in moms – to 1 decimal place Normal Result – code as	 777
	AMNIO	Was an amnio carried out?	Yes No CVS	1 0 2
	GESTAM	How many weeks gestation at amnio?	Code as number of completed weeks	
	AMNIOAFP	Amniotic fluid AFP result	Code in moms – to 1 decimal place	
	KARYO	Karyotyping results	Normal Abnormal	1 2
D.	HEIGHT	Mother's height	Code in cms	
	WEIGHT	Mother's pre - pregnancy weight	Code in kgs (to one dec pl)	
E.	COFFEE1 COFFEE2 COFFEE3	Cups of coffee consumed daily during 1st, 2nd & 3rd trimesters	Code number of cups of caffeinated coffee per day for each trimester Small cup=1 Mug=2	
	TEA1 TEA2 TEA3	Cups of tea consumed daily during 1st, 2nd & 3rd trimesters	Code number of cups of caffeinated tea per day for each trimester Small cup=1 Mug=2	

COLA1 COLA2 COLA3	Cups of cola consumed daily during 1st, 2nd & 3rd trimesters	Code number of cups of caffeinated cola per day for each trimester Small glass=1 Large glass=2 (ie half pint) Can (330ml) 1 pint (568 mls)= 4 1 litre = 8

Appendix G: Protocol for the Preparation of Hair Samples for Analysis

PROTOCOL FOR THE PREPARATION OF HAIR SAMPLES FOR ANALYSIS

Two step extraction involved:
(1) Extraction in methanol
(2) Solid phase extraction (SPE)

Extraction in Methanol

Standards

50mg of blank hair will be spiked with different concentrations of drug and extracted in the same way as the samples.

N.B. Internal standards will be added to all hair standards and hair samples (Conc. of 100ng)

Method

1) To each standard/unknown/QC hair sample, add 4ml methanol.
2) Add 50ul of Internal standard.
3) Wrap parafilm around top of each tube.
4) Incubate in water bath at 45°C for 18-24 hrs.
5) Remove methanol and transfer into a separate tube.
6) Dry down methanol and reconstitute in 3 ml of 0.1M Phosphate Buffer and mix. Sample ready for SPE.

SPE protocol using Narc-2 columns

1) Condition Narc-2 column with 3ml methanol followed by 3 ml deionised water and then finally 3m phosphate buffer.
2) Load sample onto column and set flow rate to about 1ml/minute. Collect eluent into a separate tube and save for THC extraction.
3) Wash column with 3ml water then 3ml 0.1M HCL and then finally 3ml methanol.
4) Leave vacuum on and allow columns to dry for 5 minutes.
5) Elute twice with 2.5ml of elution buffer.

Elution Buffer: Chloroform: isopropanol: Ammonia (80:20:3). Make up fresh buffer each time.

SPE protocol using Narc-1 columns (for cannabis screen)

1) Condition columns with 2ml methanol followed by 2mls 5% methanol in 0.1M acetate.

2) Load sample collected from previous SPE extraction.
3) Pull through column at minimum vacuum.
4) Wash columns with 60% methanol in water.
5) Dry columns thoroughly.
6) Elute with 2mls Hexane/ethyl acetate/acetic acid.

Derivatisation

1) Combine 2 fractions into one tube and dry down in heating block under Nitrogen.
2) Reconstitute in 200ul of ethyl acetate and transfer to autosampler vials.
3) Dry down and derivatise with 10ul pyridine, 30ul of MBTFA and 5ul of MSTFA.

N.B. wash syringe between each addition with absolute alcohol and hexane.

4) Heat in hot block for a minimum of 30 minutes at 70°C.

Reagents to be made up:

1) 0.1M HCL

2) 0.1M Phosphate buffer at ph6.

3) 5% methanol in 0.1M acetate.

4) 60% methanol

5) Hexane/ethyl acetate/acetic acid

Appendix H: Cut-Off Values for RIA and LC/MS-MS Hair Analysis

Second analysis of hair samples gastroschisis study

All hair samples for the second analysis will be analysed using radio-immunoassay (RIA) analysis.

The cut-offs employed in the immunoassays are shown below in Table A. These are based on a 50 mg hair sample

Table A: Cut-off values used for various recreational drugs in the radio-immunoassay analysis.

Test	Higher Cut-off	Lower Cut-off	Recommended Cut-off [1]
Cocaine	0.5 ng/mg	0.2 ng/mg	0.5 ng/mg
Opiates	0.2 ng/mg	0.1 ng/mg	0.5 ng/mg
Methadone	0.2 ng/mg	0.1 ng/mg	n/a
Amphetamines	1.0 ng/mg	0.2 ng/mg	0.5 ng/mg
Benzodiazepines	0.5 ng/mg	0.2 ng/mg	n/a
Cannabanoids (THC)	0.05 mg/ng	0.02 mg/ng	n/a

No specific cut-offs were employed for methylamphetamines - results obtained by comparison to blank hair.

The methamphetamine immunoassay was shown to be unreliable. Cross-reactivity was occurring with naturally occurring compounds normally present in hair or possibly with compounds present in shampoos etc. (e.g. trimethylammonium salts). The amphetamine immunoassay was therefore used as a preliminary screen for both amphetamines and methylamphetamines.

Definition of test results

Negative = below the lower cut-off
Positive = above the higher cut-off
Borderline = between the lower and higher cut-off.

All samples that have positive or borderline- positive results (with the exception of cannabanoids) will be analysed by LC-MS/MS to confirm results and quantitate levels. There is no confirmatory assay available for cannabinoids (THC) by LC-MS/MS.

The limits of detection for the LC-MS/MS analysis are shown in the Table B. These limits are dependent upon the weight of the hair sample for analysis.

The recommended limits of detection are the minimum amounts that the chromatographic test used (either LC-MS/MS or GCMS) should be able to quantify. These limits are recommended by the Society of Hair Testing[1].

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Table B: Cut-off values used by hair weight for various recreational drugs and their analytes in the LC-MS/MS analysis.

Analyte	Per sample	Expressed as ng/mg weight of hair				Recommended minimum detection limit
		10mg	25mg	50mg	100mg	
Cocaine	2 ng	0.20	0.08	0.04	0.02	<0.50 ng / mg
Benzoylecgonine	2 ng	0.20	0.08	0.04	0.02	<0.05 ng / mg
Morphine	2 ng	0.20	0.08	0.04	0.02	<0.02 ng / mg
6-MAM	2 ng	0.20	0.08	0.04	0.02	<0.02 ng / mg
Codeine	2 ng	0.20	0.08	0.04	0.02	<0.02 ng / mg
Amphetamine	4 ng	0.40	0.16	0.08	0.04	<0.02 ng / mg
Methamphetamine	4 ng	0.40	0.16	0.08	0.04	<0.02 ng / mg
MDMA	2 ng	0.20	0.08	0.04	0.02	<0.02 ng / mg
Methadone	2 ng	0.20	0.08	0.04	0.02	n/a

[1] Recommendations for hair testing in forensic cases. Forensic Science International 2004 in press.