Spatial-temporal analysis of endocrine disruptor pollution, neighbourhood stress, maternal age and related factors as potential determinants of birth sex ratio in Scotland

Ewan McDonald

Doctor of Philosophy

Department of Nursing Midwifery and Health, University of Stirling

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Abstract

Background: The human secondary sex ratio has been the subject of long-standing medical, environmental and social scientific curiosity and research. A decline in male birth proportion in some industrialised countries is linked to endocrine disruption and is validated by some empirical studies. Increasing parental age and population stress and associated decreases in sex ratio have also been demonstrated. A thorough literature review of 123 relevant and diverse studies provides context for these assessments.

Methods: A spatial-temporal investigation of birth sex ratio in Scotland and potential determinants of endocrine disruptor pollution, socio-economic factors including neighbourhood stress, deprivation, smoking, and maternal age, was conducted. This involved review of national and regional sex ratio time trends, and stratified/spatial analysis of such factors, including the use of GIS tools. Secondary data were sourced from Scottish Government web portals including Scottish Neighbourhood Statistics and the Scottish Environmental Protection Agency.

Results: Regional differences in sex ratio between 1973 and 2010 are observed which likely lever the national male birth proportion downwards, with the region of poorest air quality from industrial emissions, the Forth Valley, displaying the greatest sex ratio reduction. Further analysis shows significant upwards skewing in sex ratio for the population cohort experiencing the least and 2nd most deprivation. Localised reductions in sex ratio for areas of high modelled endocrine disruptor pollution within the Central Region in Scotland are also displayed.

Discussion: Limitations of the analyses include the danger of ecological fallacy in interpreting from area-based measurement and the simplified pollution modelling adopted. Despite this, and given elevated incidence of testicular cancer in Scottish regions mirrors the study's results, tentative confirmation of the endocrine disruptor hypothesis can be substantiated. Further, elucidation on advanced parental age as a contributory factor to secondary sex ratio change is also given. Recommendations are made with respect to environmental monitoring and health protection, and preventative health strategies in Scotland.

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Chapter 1: Introduction

The human secondary sex ratio (proportion of live male births from total live births) has been the subject of long-standing medical, environmental and social scientific curiosity and research. It can also serve as a useful demographic and reproductive health marker in populations. The over abundance of male babies in India and China and skewed sex ratios owing to sex selective abortion practices is a conspicuous observance of such a measurement. In Western industrialised countries there is speculation surrounding a relatively small decline in proportion of male births, chiefly post World War II, and whether this trend indicates 'sentinel' reproductive health changes for selected populations. According to some commentators this can be primarily attributed to pollution; specifically chemical agents which can disrupt human and animal hormones and reproduction known as endocrine disruptors (Davis, Gottlieb et al. 1998; Van Larebeke, Sasco et al. 2008).

The reduced male births observance is affiliated with other 'sentinel changes' in human health associated with endocrine disruption, that of declining sperm quality and increasing incidence of testicular cancer and male reproductive disorders, the so-called 'emasculation of men' (Davis 2002; Daniels 2006; Van Larebeke, Sasco et al. 2008). Additionally evolutionary biological and psychological hypotheses are presented as influencing sex ratio with particular focus on the observed normal male excess of births in the human population. These include the adaptive Trivers-Willard hypothesis that predicts 'stronger' and higher socio-economic status parents bear sons, and the maternal dominance hypothesis of female ovarian control over sex determination via maternal testosterone levels and dominance personality traits (Trivers and Willard 1973; Grant

1998). Further, mechanistic hypotheses of sex determination are offered including the parental hormonal balance of testosterone and gonadotrophin, timing of intercourse, and genomic imprinting and changes in embryogenesis (Boklage 2005; James 2008; James 2009). At the macro population level and with reference to these hypotheses in many cases, other factors and agents researched are multiple and diverse, including population stress, climate change, diet, socio-cultural behaviour, socio-economic status, electro-magnetic and nuclear radiation, contraception and smoking.

The thesis elucidates the phenomenon of human secondary sex ratio and a potential recent decline in industrialised countries by undertaking a comprehensive review of such knowledge and related research. This is followed by an original investigation of birth statistics and other relevant secondary data-sets in Scotland. The work adopts a spatial temporal focus by analysing area-based data of socio-economic, demographic and health-related factors whilst also utilising Geographical Information Systems (GIS) tools to model endocrine disruptor pollution. The research project consists of the following four stages:

(i) a scoping study of studies linked to secondary sex ratio which includes an extensive literature review of potential socio-economic, environmental and/or health related determinants as well as a brief narrative review of constituent elements, mechanisms and indicators affecting the sex ratio e.g. X:Y sperm chromosomes, miscarriage;

(ii) an assessment of historical and regional birth sex ratio trends in Scotland;

(iii) stratified analysis of secondary sex ratio in small areas with prominent potential explanatory factors derived from the two reviews including socio-economic status, neighbourhood stress, maternal age and maternal smoking;

and

(iv) a spatial analysis and display, utilising ArcGIS, of small area birth sex ratio in Scotland and the Central Region, incorporating modelling of endocrine disruptor air pollution from point-source emissions data for the Central Region.

This PhD submission aims to make a unique contribution to medical, environmental and social scientific scholarship at a number of levels. Firstly, it is, as far as known, the only inquiry on secondary sex ratio to explicitly adopt an *interdisciplinary* approach for elucidation of the phenomenon. The vast collection of studies on the human birth sex ratio over many years includes research utilising multiple and different methodologies and ontological perspectives. An epidemiological project which incorporates bio-markers and serum measurement is immutably dissimilar in method to a study on biographies of prominent women in society and the sex of offspring they produce. From the outset this investigation is focused and centred on the human birth outcome and phenomenon itself rather than 'rigid' subject disciplines with their own tried and tested methods.

Although the analyses rely on geographical data and method, the overall framework for the study is for a holistic examination of factors potentially responsible for male birth proportion decline. Van Larebeke, Sasco et al. (2008) comment on the need for multi-disciplinary research for localised reproductive health events such as sex ratio skewing. Use of area-based measures and GIS tools permits analysis of both scientific and social scientific parameters. Secondly, this is an original study for analysing small area-based data in this context and is a first sex ratio inquiry for *all* of Scotland, in terms of examining socio-economic and health-related factors. The thesis makes extensive use of area-based public health information that exists for Scotland, a unique collection of data-sets developed as a public policy response to the relatively poor health outcomes common to its population. Further, the long-standing measure of urban poverty and socio-economic status in U.K. used as secondary data, the multiple deprivation index, is another distinction to this study. Thirdly, by combining environmental and socio-economic/health-related area-based variables, the study aims to advance research on environmental and social epidemiology in the field of reproductive health science.

The initial task of the research was to identify previous relevant studies which investigated birth sex ratio determinants in populations. A *scoping* review was chosen as the method for accomplishing this, in preference to undertaking a standard systematic review. Whilst the rigour and depth of a systematic approach may yield significant findings the topic is ostensibly *exploratory* and methodologies across disciplines can be difficult to qualitatively differentiate. The scoping method of retrieving and considering evidence can be used to establish the range and nature of the research activity, potentially indicating whether a systematic review is feasible (Arksey 2005). For this thesis, appraisal of the validity and reliability of study methods also forms part of the sex ratio inquiry scoping review. An on-going process of reviewing and a full systematic review of the subject may result from carrying out this 'scoping' section of the study.

In Chapter 2 the first part of the review is presented, with searches of the MEDLINE, Environmental Sciences and Pollution Management, and Social Sciences Citation bibliographic indexes. 6 different sex ratio search terms are used, including 'secondary sex ratio', 'male birth proportion', and male: female birth ratio'. 3448 articles were identified of which 123 were finally selected for appraisal from applying eligibility criteria. Research on environmental and socio-economic determinants also form the first part of the review in Chapter 2.

The 'evidence' based foundation of any systematic type review though does have limitations. Deducing statistical relationships amongst sampled and national populations to infer 'cause and effect', or *the* variable or phenomena which produces a change, is not the only approach to understanding the scientific and social world. In the second section of Chapter 3 a small narrative review is undertaken, which seeks to identify generative mechanisms for adjustments in sex ratio at certain reproductive stages. For secondary sex ratio, such mechanisms, encompasses a range of reproductive and perinatal subjects which can include percentage of XX and XY chromosome spermatozoa, occurrence of miscarriage, and survivability of male or female babies at birth. These measurements and indicators are discussed with brief reviews of studies on possible environmental, health-related and socio-economic determinants. A summary of scientific knowledge on human sex determination is also given together with diagrammatic representation of the potential 'alteration' stages in spermatogenesis, fertilisation and pregnancy to produce a final live birth sex ratio in a given population. Following discussion of potential 'generative mechanisms' concluding remarks are given on both review sections with emphasis on endocrine disruptor pollution, population stress, parental age and reproductive technologies and medical intervention as *most likely* factors affecting secondary sex ratio which require further investigation.

For the Methods chapter (4), on the basis of the scoping review findings and available population data in Scotland, null hypotheses are generated. The methods and tools applied to test the hypotheses, involving a three pronged analysis, are then summarised and discussed. The data to be examined are firstly described. The Scottish Government and associated agencies produce an extensive array of socio-economic, demographic and public health data of small population areas (amalgamations of neighbourhood census zones) in Scotland, together with statutory reporting of pollutant releases in accordance with EU environmental policy directives. Indicators and measurements from secondary data-sets, including Scottish Neighbourhood Statistics Advanced Reporter, Scottish Index of Multiple Deprivation and the Scottish Pollutant Release Inventory, were selected as the independent and GIS formatted variables to test hypotheses on secondary sex ratio (the dependent variable) in Scotland and for a central regional area.

Secondly, appraisal of the quality of data to be used is given, in particular regard to validity and reliability. The accuracy and historical continuity of Scottish birth registration is viewed as a distinct advantage to the study, particularly the geo-coding of sex at birth to small areas, known as *datazones*, which are concomitant with health and socio-economic area measurement. Specific socio-economic indicators assessed on the basis of data quality include: Scottish Index of Multiple Deprivation (SIMD); percentage of women smoking at first ante-natal booking; percentage of women aged 35 and over at first birth; percentage prescribed drugs for Depression, Anxiety or Psychosis; No. of SIMD Crimes per 1000 persons, and Hospital Admissions for Drug Misuse. The latter 3 indicators are combined to produce a proxy measurement of neighbourhood stress. The accuracy of SPRI emission data, produced by the Scottish Environmental Protection Authority, is also discussed in this section of the chapter. Thirdly, the 3 stages of the comprehensive analysis of birth sex ratio are summarised and evaluated, sex ratio trend display and analysis, stratified analysis of potential factors, and GIS spatial projection of endocrine disruptor pollution and secondary sex ratio. Attention is given to advantages of the spatial-temporal methodological approach adopted; offering key advantages by including social scientific and scientific parameters for investigation and *both* birth sex ratio temporality and spatial distribution. The required data processing is also outlined with comment on the efficacy and validity of the basic modelling of SPRI endocrine disruptor pollution undertaken for stage three of the analysis. Finally ethical considerations and data management are discussed in this chapter.

Chapter 5 is the Results chapters and presents statistics, tables, graphs and maps of findings including; (i) temporal and regional trends of birth sex ratio in Scotland, (ii) tests of association and stratified analysis of small area sex ratios with equivalent area-based measurement of deprivation, neighbourhood stress, maternal age and maternal smoking (iii) GIS spatial analysis of birth sex ratio across Scotland using population centroid plots and of a case study area, Central Scotland, which also incorporates modelling of endocrine disruptor air pollution.

In Chapter 6, discussion on the implications of the results in the context of previous research conducted is presented. Each of the 5 tested variables or factors (stress, age, smoking, deprivation and pollution) is deliberated on with final conclusions on the likely influence of each on secondary sex ratio in Scotland. Limitations attached to the methods adopted in this study are considered together with further appraisal of relevant research on sex ratio and reproductive health parameters.

To conclude, in Chapter 7, comment is made on implications for research disciplines, such as environmental health and peri-natal epidemiology, as well as public health policy, notably reproductive and peri-natal health interventions. A proviso is given though on ‘over-reaching’ implications arising from research findings with discussion of some of the methodological weakness associated with the type of study conducted.

By adopting an interdisciplinary approach the simple purpose of the research is to unpack some of the mystery surrounding determinants of the secondary sex ratio and more particularly its recent declining trend in industrialised countries. The thesis aims to make an important contribution to understanding reproductive health in Scotland and beyond, whilst also adding to scientific debate on possible endocrine disruption human effects.

Chapter 2: Potential Determinants of Birth Sex Ratio Change in European and North American populations: Scoping Review Part 1

2.1 Introduction: Scoping Review – Part 1

The following two chapters seek to synthesize evidence of key potential factors influencing sex ratio decline in industrialised countries by presenting an extensive scoping review of relevant research. The objective of such a review (scoping) is to document the 'extent, range and nature' of knowledge on a given topic (Arksey 2005); for this case *birth sex ratio change in European and North American populations*. The thesis draws on a range of disciplines in both the sciences and social sciences. For this reason, the scoping exercise is unusually substantial, as it needs to cover, and be informed by, several bodies of knowledge.

The major component of the assessment in the two chapters is a thorough literature review of 123 observational studies of human sex ratio and potential explanatory factors, which is presented over both chapters. A systematic approach to identifying related studies and assessing the research quality is adopted. In addition, a brief narrative review of potential generative mechanisms involved in sex ratio change, focusing on associated birth and reproductive health indicators, forms part of the overall scoping review. Theory and evidence synthesised from both the large literature and small narrative review is the applied to formulating key research questions for this thesis. Chapters 2 and 3 thus serve as a substantial introduction to the subject as well as documenting extensive evidence from which to generate hypotheses for testing. The scoping review is composed of two parts. Chapter 2 contains Part 1 of the review covering methods for the systemtically-formed review, including scope and protocols, eligibility criteria, search strategy, critical appraisal, and results on environmental and socio-economic influences. Chapter 3, with Part 2, details health-related factors for results of the literature review, a relevant but small narrative review of reproductive mechanisms and indicators, and a final synthesis and discussion for the whole review with emerging research questions. An outline of background knowledge on mammalian sex determination and a précis of human secondary sex ratio study, however, are firstly given to set the scene.

2.1.1 Background: Trends and Definitions

The phenomenon of a declining proportion of male births in many industrialised countries, over approximately the last 60 years, has been widely documented. Statistical studies indicate a trend occurring in the Netherlands, Sweden, Denmark, Canada, U.S.A., Japan, England and Wales, Greece, Germany and Spain (Moller 1996; Allan, Brant et al. 1997; Dickinson and Parker 1997; Martuzzi, Di Tanno et al. 2001; Davis, Webster et al. 2007; Tragaki and Lasaridi 2009). Although no meta-analysis of individual country studies has been undertaken, one study which analyses World Health Organization data reports a significant overall decline in male births across Europe and North America (Grech, Vassallo-Agius et al. 2003). A further study, adopting a global perspective, shows male birth proportion decrease confined to developed countries, with most observed in northern and eastern Europe (Parazzini, La Vecchia et al. 1998).

This description of the male proportion of births is more commonly referred to as the *secondary sex ratio* and is calculated by dividing the number of male live births by the total number of live births (male + female). A male excess of 106 babies for every 100 female babies, a sex ratio of 0.515, is established as the given global average, with some of the aforementioned reductions ranging from 0.515 to 0.513 over a 30 to 50 year period. This review appraises research seeking to explain such a phenomenon as well as relevant earlier studies which examine factors influencing increasing or decreasing sex ratio in populations.

2.1.2. Background: Biological Mechanisms, Primary Sex Ratio and Secondary Sex Ratio

Understanding biological mechanisms determining human sex ratio is fundamental to appraising the selected research from the review. Mammalian sex determination has been the subject of much scientific investigation and sometimes absurd conjectures for centuries. Aristotle promulgated the theory that sex was determined by the body temperature of the male partner at conception (Gilbert 2000). For over a thousand years misogyny in philosophy and anatomy prevailed with the hypothesis that females were inferior, poorly developed males. It was not until the 17th century that female anatomy of ovaries and both sex organs were studied in physiology (Gilbert 2000). Established knowledge on reproductive human physiology now states that it is the XY genetic male or XX genetic female chromosomes from paternal spermatozoa which determine sex during conception and fertilisation. However, some scientists have hypothesised that it is the maternal ovum which is 'attracted' to either type of chromosomes from male sperm (Grant 1998). An important breakthrough in genetics was also made more recently with the discovery of the SRY gene (sex determining region on the chromosome Y), effectively 'the master switch' in human sex determination (Kashmida 2010).

A rudimentary summary of the biological process of human reproduction is therefore as follows: Following sexual intercourse, the man's XX (female) or XY (male) bearing spermatozoa fertilises, or is fertilised by [1], the oocyte (immature ovum) in the woman. The percentage of fertilised XX or XY spermatozoa at the time of conception is known as the *primary sex ratio*. Sex-specific gonadal development is then triggered by SRY expression, with Sertoli cell production from XY genital ridges, leading to Leydig cells and testis development in the embryo. In the absence of Sry in XX genital ridge, other genes such as Wnt4 and Foxl2 initiate follistatin which differentiates granulosa and theca cells which eventually form ovarian follicles in embryonic development (Kashmida 2010). This sex differentiation in the womb coincides with the time of recognised clinical pregnancy (6 – 8 weeks gestation).

The eventual secondary sex ratio and usual excess of male births can be linked to not only the primary sex ratio and embryogenesis in the womb but also survivability of the sexes over the entire pregnancy. Through citing clinical evidence and inferring from zoological and veterinary studies, biological theories have been offered on determination of the human primary sex ratio and mechanisms antecedent to it. A key observation informing such hypotheses is that when foetal loss does occur, male embryos are disproportionately lost. This is also reflected in later pregnancy with a similar imbalance of peri-natal deaths(Hassold 1983; Zeitlin, Saurel-Cubizolles et al.

[1] Locus of control for fertilisation, either male or female, remains controversial. Refer Rosenfeld, C., ; Roberts, R.; (2004). "Maternal Diet and Other Factors Affecting Offspring Sex Ratio: A Review." Biology of Reproduction **71**: 8.

2002; Vatten and Skjaerven 2004) Given this, some scientists have claimed that the primary sex ratio is significantly higher than the final secondary ratio of 105/106 male to 100 female live births. Measurement of the primary sex ratio however is highly invasive and ethically prohibitive and sperm sex ratio, the proportion of XX and XY bearing chromosomes in ejaculated spermatozoa, is a frequently referred to as a possible proxy. Some studies have shown differences in proportion of XX and XY bearing sperm according to father's siring of only sons or only daughters, as well as reduced sexual abstinence (Lazarus 2002). Extrapolating from these and animal studies one hypothesis portends that it is the balance of hormones testosterone and gonadotrophin for couples which determine the sex ratio at conception and fertilisation, and this is further linked to the frequency of coitus and timing of it in the menstrual cycle (James 2008). The surge in luteinizing hormone (LH) produced by gonadatroph cells in the middle of the menstrual cycle is pivotal to the hypothesis with female births most likely when conceptions occur at this time.

The hypothesis however is challenged by contradictory evidence from previous randomised control trials in artificial insemination as well as consistent reports that the sperm sex ratio is ostensibly 50% XX to 50% XY (Grant 1998; Graffelman, Fugger et al. 1999; Boklage 2005). A competing theory of secondary sex ratio determination is that of differential survival of sexes in embryogenesis. The adjustment to male excess occurs not at the stage of fertilisation (i.e. primary sex ratio) but during embryo formation whereby excess loss of female embryos occurs as a result of sex differences in genomic imprinting. Potentially problematic for this mechanistic theory, however, is the contradictory process of preferential loss of male foetuses throughout pregnancy though an early ‘strength’ to male embryo formation followed by greater fragility is at least a feasible model to investigate further.

The vulnerability of the male foetus to spontaneous abortion and peri-natal death is a key observation for secondary sex ratio research. There are a number of potential factors responsible including evolutionary predisposition to male foetal loss and/or a stress response in pregnancy with an increase in maternal corticosteroids in the 20th gestational week (Catalano 2003; Bruckner, Catalano et al. 2010). Debate in genetics and cellular developmental biology is also noteworthy to male foetal vulnerability, with the possible degeneration of the male Y chromosome in nature a potential contributory factor. By virtue of its small size and paucity of associated genes, the human Y chromosome degrades rapidly according to comparative studies with invertebrates, insects and mammals (Graves 2006). Possible reasons for this are also intriguing, particularly in the context of epidemiological trends of declining male reproductive health. The greater cell divisions required to make sperm compared with an egg results in more opportunities for damage and sperm is also an ‘oxidative environment’ which ‘lacks repair enzymes’ (Aitken 2002).

The primary sex ratio can therefore be adjusted from mechanisms occurring in foetal development, pregnancy and birth which are considered in some of the studies identified and reviewed as well as in the next chapter which also includes a small narrative review on the subject. Significantly, such mechanistic theory indicates that secondary sex ratio is intrinsically linked to both *male* and/or *female* reproductive physiology and health, which in turn can be influenced by a multitude of environmental, socio-economic, demographic or behavioural factors.

2.1.3. Background: Population Studies

The earliest research on aetiology and factors influencing human secondary sex ratio initially arose from biological curiosity surrounding evolution and mammalian sex determination. This motivated statistical studies on parental age and socio-economic status by demographers and epidemiologists in the 1960s. The Trivers Willard hypothesis, proposed in 1973 and suggesting that parents in stronger ‘biological condition’ produce more boys, has led to many demographic analyses on socio-economic status as a proxy indicator. This also includes most recent work on testing of this hypothesis with frequently large data-sets. Investigations also focused up to 40 years ago on reproduction itself, with analysis of contraceptive use as a factor, as well as coital rates in populations.

Environmental influences on sex ratios, such as from pollution or natural disasters were generally not conducted until about the last 20 years. Williams, Lawson & Lloyd’s (1992) geographical analysis on air pollution in central Scotland, and Potashnik's (1984) occupational study on DBCP, are widely cited as pioneering studies in this regard. Since then, such research has expanded concurrently with observations of the male birth proportion decline phenomenon, with some commentators suggesting an environmental link and a 'sentinel' change in reproductive health in industrialised nations (Davis, Gottlieb et al. 1998). This fits closely with the environmental endocrine hypothesis, whereby industrial activity and the ubiquity of hormone disrupting compounds are potentially responsible for reproductive changes in humans, with such toxic effects already discovered in animal populations.

Subsequently, published articles have concentrated on debates surrounding the environmental case and investigations on other, or potentially additional, causes of sex ratio decline, such as changes in diet, maternal stress, economic contraction, smoking and reproductive technologies. This thorough literature review is uniquely inter-disciplinary, in attempting to synthesise evidence available on socio-economic, health-related and environmental factors implicated in recent sex ratio decline. The review includes relevant material on sex ratio skewing, both upwards and downwards, as well as the earlier research which pre-dates the 'sentinel' change hypothesis.

Two previous systematic reviews have also been identified in the search which includes sex ratio as one of the measurements in integrated analyses of birth outcomes (Strobino, Kline et al. 1978; Zilko 2010). These reviews focus on chemical exposures and economic contraction as influences with birth outcome variables such as small for gestational age and number of pre-term deliveries also included as peri-natal and neo-natal measurements. A review which concentrates exclusively on sex ratio as the outcome variable is therefore required and this thesis partly addresses such a gap through this and the subsequent chapter. The necessary narrower focus on such a single outcome also permits a wider analysis of causative factors, including both chemical exposures from pollution as well as socio-economic, demographic and health-related determinants.

2.1.4. Background: Epidemiological terms and Sentinel sex ratio 'thresholds'

Before evaluating previous sex ratio study it is necessary to define some of the epidemiological and methodological terms integral to this type of research. Discussion on method and validity of public health study frequently focuses on the complexity attached to reporting *associations, causality* and *confounding*. These can be defined as follows;

Association – a *statistical* relationship between two measured quantities or variables whereby the existence of one affects the probability of the other.

Cause – in the context of health scholarship and practice Rothman (1976) defines a cause as 'an event, condition or characteristic (or combination of these factors) that plays an essential role in producing an occurrence of the disease.' For the purposes of this research, the term 'disease' can be substituted for health event or health outcome.

Confounding – occurs when an alternative explanation can be given for a cause of disease or health outcome which is not explicit in the association of two variables. It is often refers to the 'mixing or muddling of effects' that can happen when looking for specific cause(s) from association(s).

The effect, or health event or outcome, for this body of work is sex ratio for a given population and/or time period, and researchers have attempted to establish the causes of such an effect(s). The difficulty arises from proving causation only on the basis of a statistical relationship or association, particularly where confounders are likely. Webb, Bain et al (2009) surmise elements for evaluating causation in epidemiological studies which include the following;

Temporality – the exposure must precede the disease, or health outcome.

Strength of association – stronger associations mean less likelihood of confounding

Consistency – the effect is found consistently across a number of studies

Dose-response relationships – level and duration of exposure is related to the extent of health outcome or disease

Biological plausibility – validity attached to likely biological mechanism explaining the cause and effect

Specificity – controversial concept whereby if association is linked to one outcome then causation is more likely.

Notwithstanding other elements, evaluating causal factors for sex at birth outcomes is particularly specific to temporality and dose-response relationships. As described above, for potential mechanisms attached to sex ratio, there are a number of temporal junctures where effects may be definitive. Further, the environmental endocrine hypothesis, examined as a potential causal factor, is predicated on a challenge to the dose-response axiom in toxicology and exposure science in presenting low-dose, teratogenic effects where timing can be a crucial element.

The extent of sex ratio variability and what constitutes alarming effects to health is also of importance for evaluating previous research. The hypothesis that male birth proportion decline in industrialised countries represents a change in population reproductive health is an example of early warning sentinel surveillance. The countries' trends described above are statistically significant declines over time, calculated by regression modelling. Similarly, statistical significance, where p-value < 0.05, is potentially then a marker of concern for any regional areas and smaller population areas and population cohorts. Simple binomial testing for example, where the normal sex ratio of 0.514 is used to assess variability across different total numbers of male and female births (N) for groups or areas, is one straightforward approach. A p-value <0.05 in such a test would therefore indicate that a sex ratio which is substantively different to the norm does *not* occur by *chance* . In any epidemiological study though, claims of cause and effect or sentinel changes from statistically significant results are exercised with caution and this is potentially even more so with analysis of sex ratio data. Invariably a simple projection of a linear trend of sex ratio over time will show strong oscillations and a somewhat irregular pattern. This is because sex ratio can be categorised as a *stochastic* variable such that its determination or value is composed partly by random. An analogy can be drawn with stock market and climate trends as 'variables' which similarly lack predictability. Skewing of sex ratios from the normal range which are statistically significant may therefore be more likely attributed to chance than other health outcomes with more stable counting, such as low weight births or cancer incidence. Sentinel surveillance of sex ratio for reproductive health thus involves comprehensive assessment of trends, both significant and non-significant, across substantive time periods, geographical areas and population groups. Associated reproductive health indicators, such as sperm quality and/or peri-natal deaths, may also form a package of epidemiological data together with sex ratio skewing that provides a signal for human health impacts.

2.2 Literature Review: Methods

The rest of this chapter and the first part of Chapter 3 cover the literature review of studies on potential determinants of birth sex ratio in European and North American populations. This section details the methods adopted, including scope and protocols, eligibility criteria, search strategy and critical appraisal.

2.2.1 Scope and Protocols

This review follows principles and protocols from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, detailed in Appendix A. Further guidance on structure and method was also obtained from social science sources and a public health systematic review (Egan, Petticrew et al. 2003; Petticrew 2006). A major systematic review involves a thorough quality appraisal of a discrete number of identified studies from a clearly defined search. Such an approach is beyond the confines of this doctoral work, particularly given the exploratory and broadly defined nature of the study. This review, however, which adopts principles from the aforementioned academic method, serves as a scoping review of the study of, and evidence for, changes in sex ratio and possible explanatory factors. Systematic reviews of these particular influences in discrete subject areas, method types or population groups may be an avenue for further research.

2.2.3 Eligibility Criteria

English language articles were searched for that included the terms; 'sex ratio', 'secondary sex ratio', 'male birth proportion’, 'male birth fraction' and 'male: female ratio'. Articles selected, included those which addressed the question of factors influencing male birth proportion decline as well as analysis of variables which may be associated with both increasing and decreasing sex ratio change. Much research was conducted preceding the male birth ‘decline’ debate and studies showing upward sex ratio alteration can add to knowledge on mitigating factors affecting the recent male birth proportion declining trend.

The large volume of studies on upward sex ratio skewing in South East and East Asia as a result of common sex selective abortion is excluded from the review. The declining male births trend is experienced predominantly in Western countries and therefore research specific to populations in Asia is not applicable to the parameters of this review. Although studies exist on South Asian immigrant populations in Canada and the UK, reporting only significant changes in female birth proportion for particular years and Indian born mothers, the numbers affected is very small within North American and European countries (Hesketh and Xing 2006; Dubuc and Coleman 2007; Auger, Daniel et al. 2008).

A prolific topic in birth ratio research is also the observed trend of increased male births during and immediately after major wars, particularly the Second World War. Given the absence of major conflict in the affected industrialised countries in the last 60 years, militarised warfare over a long period as a factor was excluded from the review. The presence however, of acute ‘stressful’ violent events such as terrorist attacks and short outbreaks of warfare is included in the review as such investigations are explicitly examined as proxy indicators for population stress.

Case control studies of sex ratio changes linked to medical aetiology, such as muscular dystrophy and testicular cancer, also emerged from searches conducted in the review. Increased incidence of testicular carcinomas in industrialised countries is hypothesised as one component of testicular dysgenesis syndrome and the deterioration in male reproductive health potentially from endocrine disrupters and pollution (Skakkebaek, Rajpert-De Meyts et al. 2001) Given this possible link, any empirical research on such cases is relevant to the environmental endocrine hypothesis and a possible connection with the male births decline phenomenon. Muscular dystrophy cases, however, are not directly relevant to the hypothesis and the affected populations are too small for consideration to be included in the review. New reproductive technologies such as artificial insemination and contraception were included in the review, although comparative clinical studies focusing on particular fertilisation techniques were not selected. Physical geographical features, such as latitude and altitude were also not included in the review.

The ranges of study designs included in the review were all quantitative, comprising cohort and case-control studies, ecological and historical population studies, geographical analyses and meta-analyses. Studies which solely analysed sex ratio trends and then offered suggestions of causes were excluded from the review, except where small, discrete populations were affected by an obvious, consistent variable, such as pollution or natural disaster. Research selected therefore contains a methodological framework which comprises statistical analysis of the dependent variable of secondary sex ratio and one or more independent variables, the potential determining factors. Commentaries or letters solely considering particular mechanistic theories of sex ratio were therefore excluded from the review, though such discussion is occasionally referred to on examining the validity of some of the selected studies.

2.2.4 Search Strategy

Factors influencing sex ratio are associated with a wide variety of disciplines, including environmental science, toxicology, demography, public health and medical anthropology. Given the inter-disciplinary nature of this research, three different subject databases with comprehensive published literature formed part of the review. MEDLINE, the largest collection of published medical literature, was searched from 1966 to 2010. A search was also conducted of the Environmental Sciences and Pollution Management databases from its earliest records, 1967, up to 2010. The Social Sciences Citation Index on the Web of Knowledge portal was also searched from 1966 to 2010.

A PRISMA Flow Diagram for this review is shown in Figure 1. A total of 3441 titles and abstracts were initially retrieved of which there was considerable duplication across the databases. An earlier scoping of published literature produced another 24 articles obtained from bibliographies of key articles.

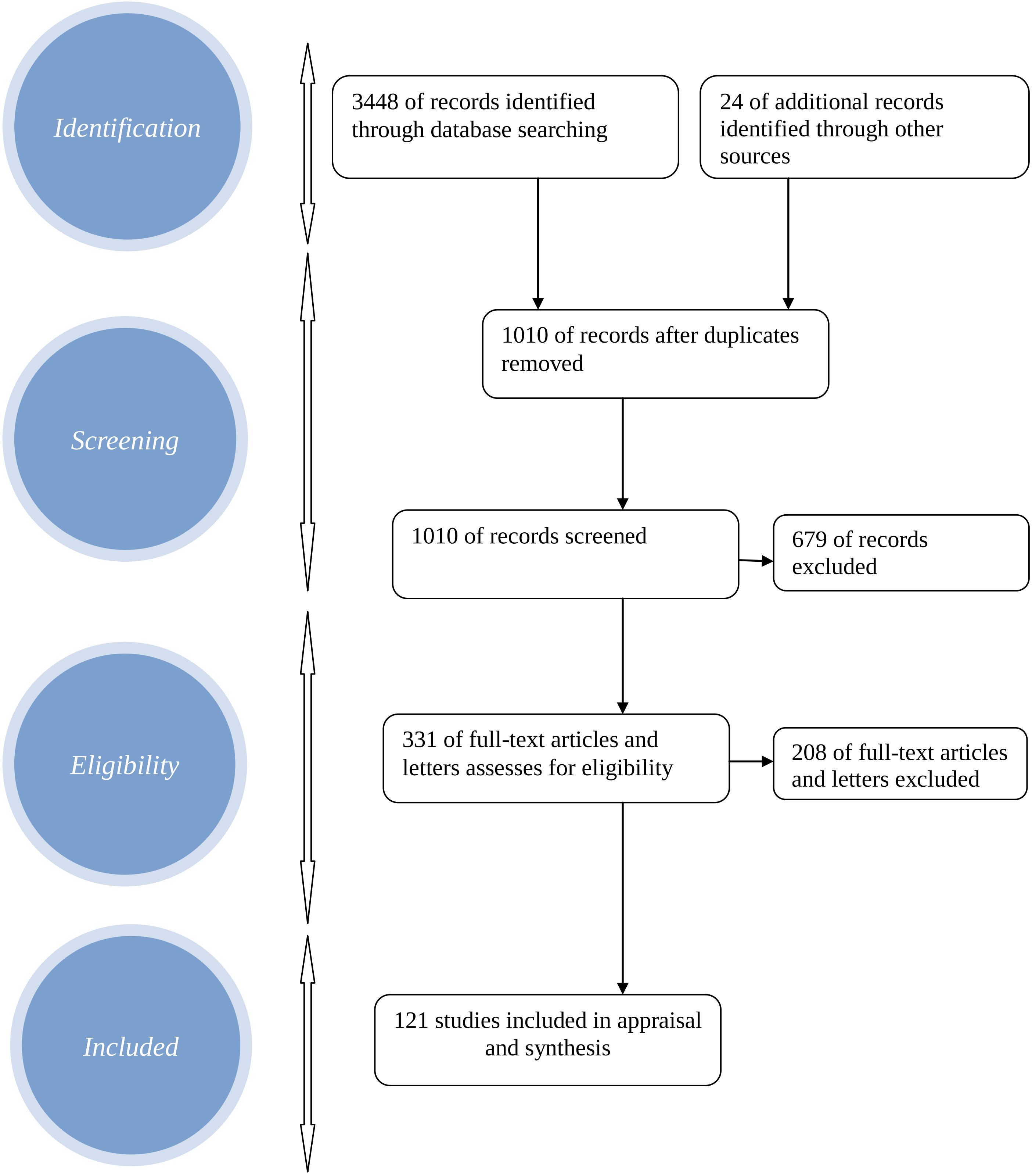
After screening for the eligibility criteria described above, 328 full articles, letters and abstracts were selected. These studies and correspondence were then further assessed for their particular relevance and the strict methodological criteria outlined above. A total of 123 articles and letters were finally included for appraisal and synthesis.

2.2.5 Critical Appraisal

Although studies included methodological approaches not specific to epidemiology, such a statistical trend analysis in demography and toxicology measurement, systematic reviews are pre-dominantly conducted within the health domain. The studies were therefore critically appraised according to common methodological criteria for epidemiological study, outlined by recognised training institutes and public health scholars (Solskone 2008; Critical Appraisal Skills Programme 2009).

The following criteria for appraising selected studies were therefore applied. Firstly, judgement was made on the theoretical and empirical soundness of factor investigated or the validity of the particular determinant investigated. This could be based on previous empirical research conducted and/or the biological hypotheses proposed. Secondly, the accuracy of how exposure was measured in the investigation was evaluated. This ranged from the use of biological markers in many toxicological investigations to suggestions of exposure amongst populations in ecological studies. Thirdly, the extent to which bias had been minimised in the study was assessed. This addresses matters of whether a cohort or case was sufficiently representative and/or whether systemic error had occurred in the results of the study. Fourthly, confounding factors were also examined including assessment as to whether other potential contributory causes had been missed and, if confounders were included, how they were accounted for. Finally, the size of the populations or cohorts investigated contributed to judgements on study quality.

Figure 1: PRISMA Diagram for Birth Sex Ratio Change Research Review



2.3 Literature Review: Results - Environmental and Socio-Economic/Demographic Factors

A total of 123 different studies were finally identified, of which 115 were from the electronic databases and 8 from citation follow-ups. The 2 summary tables below differentiate the studies according to possible exposure or potential factor involved, and the type of method used.

Table 1: Birth Sex Ratio Studies according to Exposure Type/ Potential Factor

Exposure Type / Potential Factor	***Number of Studies***
Environmental	
Dioxins	6
Polychlorinated Bi-phenyls (PCBs) and Polybrominated Bi-phenyls (PBBs)	6
Pesticides	10
Mercury	1
Lead	1
Boron	1
Nuclear Radiation	5
Electro-magnetic Fields	8
Local Pollution (Multiple Media)	6
Air Pollution	7
Socio-Economic and Demographic	
Socio-Economic Status	12
Socio-cultural or Socio-psychological	4
Parental Age	19
Health-Related	
Smoking	6
Alcohol	3
Stress	12
Diet/Nutrition	5
Reproductive Health and Interventions	11

Table 2: Birth Sex Ratio studies according to Method Type

Method Type	***Number of Studies***
Ecological	40
Cohort	42
Case Study	8
Cross-sectional	8
Case-control	10
Time-series analysis	8
Meta- analysis	3
Geographical analysis	4

Table 1 illustrates the diversity of factors and exposure types investigated with the summary categories of environmental, sociological and demographic, and health-related carrying broadly equal representation in the final totals. The environmental exposure types are also differentiated to reflect toxicological investigations of specific chemicals. Table 2 shows the majority of the methods employed include ecological studies and examinations of cohorts. Table 3 is a summary table of all 123 studies detailing the study type, size of sample, main findings and brief comment on methods used. Environmental and socio-economic/demographic factors are discussed in the next sections of this chapter, whilst presentation of results health-related determinants forms the first part of Chapter 3.

Table 3: Literature Review – Summary Table: Key

+ F / - M Significant increase in female births/decrease in male births (decreased sex ratio)
+ M / -F Significant increase in male births/decrease in female births (increased sex ratio)
= No significant association or changes to sex ratio

Table 3: Literature Review – Summary Table

Author (Year)	**Country**	**Sample size**	**Study Design**	**Exposure of interest**	**Main findings**
Mocarelli, Brambilla et al. (1996)	Italy	198 parents	Time-series	Dioxin - TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin)	+ F / - M. 9 months post large TCDD release in most contaminated zone (26 males: 48 females). Descriptive time series, tables and graphs April 1977 – Dec 1984.
Mocarelli, Gerthoux et al. (2000)	Italy	535 parents	Cross-sectional	Dioxin - TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin)	+ F / - M. Well-designed study. Bio-measurement of exposure at *time* of pollution event. Increased probability of female births associated with increasing TCDD concentrations in serum samples of fathers, particularly for fathers exposed when younger than 19 years (SSR = 0.38)
Michalek, Rahe et al. (1998)	U.S.A.	1,208 veterans	Cohort	Dioxin – TCDD (Agent Orange)	+ M / - F. Well-designed U.S. Airforce Health study (Sprayers of Agent Orange). Longitudinal bio-measurement of dioxin.
Rogan, Gladen et al.(1999)	Taiwan	137 births	Time-series	Dioxin – PCDF (Polychlorinated dibezofurans)	= Very small sample. Non-sig. excess of females born (69 girls, 68 boys) from June 1978 to March 1985 to women affected by cooking oil PCDF contamination.
Schnorr, Lawson et al. (2001)	U.S.A.	281 workers	Retrospective Cohort	Dioxin – TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin	= Estimated TCDD level at time of conception (occupational exposure from Agent Orange production)

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Yoshimura, Kaneko et al. (2001)	Japan	85 parents	Case-study	Dioxin – PCDF (Polychlorinated dibezofurans)	= Straightforward sex ratio calculation to parents exposed to accidental release in 1968 (0.513) Exposure measurement based on geographical coverage of pollution.
Hertz-Picciotto, Jusko et al. (2008)	U.S.A.	399 mothers	Retrospective Cohort	PCBs (Polychlorinated biphenyls)	+ F / - M Well designed study, including robust bio-measurement and confounders, from 1960s Child Health cohort. Significant alteration at 90^{th} percentile of PCB concentration in serum samples.
Terell, Berzen et al. (2009)	U.S.A.	865 births	Cohort	PBBs (Polybrominated biphenyls)	= Well designed study from Michigan cohort (accidental exposure to animal feed) with bio-measurement. Near sig. increase in male births (p=0.1) for combined parental PBB concentrations in serum samples above median levels.
Weisskopf, Anderson et al. (2004)	U.S.A.	381 births	Cohort	PCBs (Polychlorinated biphenyls)	+ F / - M For highest quintile of PCBs concentration in serum samples of women exposed from fish consumption. Robust study in identified area affected by endocrine disruption (Great Lakes)
Khanjani and Sim (2007)	Australia	200 births	Cohort	PCBs (Polychlorinated biphenyls)	= Well designed study across birth outcomes. No association of sex ratio with women's breast milk PCB levels

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
International POPs Elimination Project (2006)	Russia	346 women	Cohort	PCBs (Polychlorinated biphenyls)	+F / -M For higher levels of PCB congener 118 & 138 concentrations in women exposed from traditional fish/whale diet. Comprehensive reproductive health investigation in identified area of environmental hazards from bio-magnified PCB exposure.
Jarell, Gocumen et al. (2002)	Turkey	587 mother-infant pairs	Ecological	HCB (Hexachlorobenzene)	+F / -M For women with greatest exposure to HCB from episode peak (1955 to 1957). Exposure measurement based on incidence of associated skin disease.
Khanjani and Sim (2006a)	Australia	815 births	Cross-sectional	HCB (Hexachlorobenzene) and Cyclodeines	= No association of sex ratio with women's breast milk HCB levels. As per Khanjani above, robust study across birth outcomes.
Khanjani and Sim (2006b)	Australia	815 births	Cross-sectional	DDT	= Non- significant decrease in male births with increase in DDT levels. Well-designed study across birth outcomes.
Garry, Harkins et al. (2002)	U.S.A.	695 workers	Cross-sectional	Pesticides – Occupational exposure	+ M / -F For men and women applicators with non-fungicide exposure. + F / -M for fungicide exposure amongst applicators (both sexes). Well-designed study with bio-measurement and adjustment for confounders

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Ryan, Amirova et al. (2002)	Russia	227 workers	Cohort	Pesticides: Trichlrophenol & 2,4,5-trichlorophenoxy acetic acid	+ M / - F For both men and women workers with 30 times greater concentrations of TCDD in serum samples than background exposure. Well designed study with bio-measurement and adjustments for confounders
Bretveld, Hooiveld et al. (2008)	Holland	1058 male and female workers	Cross-sectional	Pesticides – Occupational exposure	= For male and female Greenhouse workers. Well-designed comprehensive reproductive health study. Sig. elevated risk for miscarriage for male greenhouse workers though no alteration in sex ratio.
Potashnik, Goldsmith et al. (1984)	Israel	30 men 89 births	Cohort	Occuapational DBCP (1,2-dibromo-3-chloropropane) exposure	+F / -M For males with impaired spermatogenesis. Very small sample though analysis of both sex ratio and sperm quality (potential mechanism) adds validity to study.
Gibson and Koifman (2008)	Brazil	308 Municipal areas (Total Births not stated)	Ecological	Pesticides – Surrogate: Agricultural toxic sales	+F / - M For 10 municipalities (out of 308) with intense agricultural activity. Blunt measure of exposure, based on volume of agricultural sales in districts
Whorton, Wong et al. (1989)	U.S.A.	46,328 births	Ecological	DBCP water contamination	= Use of DBCP levels in water used to establish 7 exposure levels across census districts. Series of weighted averages for wells in each of the Census District. Question over precise exposure measurement.

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Clementi, Causin et al. (2007)	Italy	14,6239 births	Ecological	Pesticides – Surrogate: Levels of use obtained by interviews with industry	= No significant variation in sex ratio for each of the 3 areas: high, medium and low pesticide use. Robust spatial measurement of pesticide use through primary (interviewing) and secondary data. Part of birth defects investigation.
Sakamoto, Nakano et al. (2001)	Japan	18432	Ecological	Methylmercury	+ F / - M For both pollution affected fishermen and maternal Minimata disease patients. Large retrospective samples. Exposure measured by Minimata disease medical records. No confounding factors analysed
Jarrell and Weisskopf (2006)	Mexico	1980 women	Cohort	Lead	= No association with secondary sex ratio and maternal/foetal lead levels Well-designed study with bio-measurement (inc. maternal cord blood) and adjustment for confounders.
Sayli and Tuccar (1998)	Turkey	1678 families	Ecological	Boron –3 villages with different levels of exposure based on drinking water concentrations and type of employment for population.	= Between 3 villages. Area sex ratios according to surrogate exposure measurement. No confounders analysed.
Liu, Li et al. (1984)	Taiwan	115 births	Cross-sectional	Polluted area of Jinghai County: Electronic wastes	+ F / - M For male births in Jinghai County to ‘control’ population in Tianjin (larger regional area). Simple test of difference for sex ratio with very small sample.

Mackenzie, Lockridge et al (2005)	Canada	715 births	Time-series	Heavy pollution in Sarnia, Ontario. Exposure from adjacent large petrochemical, polymer and chemical industrial plants	+ F / - M For male births from 1994 to 2003 with greatest decrease in most recent 5 years (1999-2003). Evidence of sex ratio skewing at one of most industrially polluted localities in the world (largest industrial complex in Canada)
Viel, Floret et al. (2005)	France	375078 births	Geo-statistical analysis (spatial scan)	Dioxins – Surrogate: Proximity to solid waste incineration Pesticides – Surrogate: Spraying/use in some rural areas	= Geo-analytical study utilising scan statistics to identify clusters of low sex ratio. Modifiable areal unit problem as confined to one area – communes. No exposure assessment accounting for dispersal of air pollutants. No clusters of low sex ratio detected in close proximity to solid waste incineration or rural areas with high pesticide use
Williams, Lawson et al. (1992)	Scotland	3577 births	Geo-statistical analysis (Surface smoothing)	Residential exposure to industrial air pollution	+ F / - M In areas adjacent to incineration plant releasing dioxins. Innovative use of smoothing techniques and pioneering study on sex ratios and pollution
Williams, Ogston et al. (1995)	Scotland	72 postcode areas (Total births not stated)	Ecological	Residential exposure to multiple sources of industrial pollution (12 localities)	= Geographical study with simple allocation of post-code sectors to either pollution from industrial sources or without exposure. Modifiable areal unit problem and imprecise pollution measurement. No associations between residential exposure to pollution in localities and either low or high sex ratio.

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Lloyd, Lloyd et al (1984)	Scotland	783 births	Time-series	Residential exposure to nearby steel foundry (air pollution)	+ M / F – For polluted area adjacent to steel foundry (Armadale, Scotland). Exposure measurement just on proximity.
Lloyd, Smith et al (1985)	Scotland	Approx 2,500 births	Ecological	Residential exposure to nearby steel foundry (air pollution)	+ M / F – For polluted area adjacent to steel foundry (Bathgate, Scotland). Also significantly higher mortality for lung cancer. Low wind speed modelling of air pollution with use of soil samples
Yang, Chen et al. (2000)	Taiwan	Approx 200,000 births	Time-series	Petrochemical air pollution.	= No associations between polluted localities and either low or high sex ratio. Simple proxy measures of pollution by proximal location of plants locality. No confounders analysed.
Yang, Tsai et al. (2000)	Taiwan	Years 1971 to 1996 in 2 municipalities (Total births not stated)	Time-series	Petrochemical air pollution.	= No significant alterations in sex ratio. Elevated incidence of female lung cancer. Simple proxy measures of pollution by proximal location of plants locality
Peterka, Peterkova et al (2004)	Czech Republic	600 months of male and female births in Czech Republic	Time-series	Nuclear radiation release: Chernobyl nuclear accident in 1986	+ F / M - Straight forward review of monthly, national sex ratio trends with no analysis of confounders. Significantly less male births in Czech Republic ($p<0.05$) in November 1986 suggestive of disproportionate male foetal loss in 3rd month of pregnancy

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Peterka, Peterkova et al (2007)	Czech Republic	9649 births	Spatial analysis	Nuclear radiation release: Chernobyl nuclear accident in 1986	+ F / M – In six regions where radiation dramatically increased in May 1986.Mapping of radioactive cloud accounting for concurrent rainfall at time of event. No confounders analysed and foetal death statistics do not corroborate with findings.
Scherb and Voigt (2007)	Czech Republic Denmark Finland, Germany Hungary Norway Poland Sweden	2.474 million births	Time-series	Nuclear radiation release: Chernobyl nuclear accident in 1986	+ M / F - Time trend modelling with fall out measurements included for districts of Germany. Positive association between increase in male births and radioactive exposure at district level (odds ratio 1.0145 per mSv/a radiation) 1986 to 1991
Mudie, Gusev et al (2007)	Kazakhstan	11,464 births	Ecological	Chronic nuclear radiation exposure: Nuclear testing in Semipalatinsk region 1949-1989	= No significant association between radiation exposure level and sex ratio. Well designed study including confounders of parental age, education level and birth order. Maternal radiation measured by proxy background levels per year in region
Dickinson and Parker (1994)	England	260060 births	Retrospective cohort	Occupational exposure to nuclear radiation	+ M / F - For partners of men employed who received greater than 10mSv external radiation in 90 days before conception Very large cohort study but no confounding analysis and exposures estimated. Younger age distribution though may be explanatory factor

Maconochie, Roman et al. (2001)	England	46000 births	Retrospective cohort	Occupational exposure to nuclear radiation	= For sex ratio from exposure to low-dose chronic exposure to ionising radiation Large cohort study with exposure measurement from dosimetry records.
Baste, Riste et al. (2008)	Norway	10497 men	Retrospective cohort	Occupational exposure to electro-magnetic fields: Naval personnel	+ F / M - Well-designed large cohort study with many confounders analysed and occupational coding of exposure (e.g. high degree of working close to high-frequency aerials)
Irgens, Kruger at al. (1997)	Norway	1.2 million birth records	Ecological	Occupational exposure to electro-magnetic fields: Multiple job categories	+ F / M - For women in electro-magnetic fields industries Simple calculation of sex ratios for separate job categories. No confounders included.
Larsen, Olsen et al. (1991)	Denmark	316 pregnancies	Case-control	Occupational exposure to electro-magnetic fields: Physiotherapists	+ F / M - For women highly exposed to electromagnetic radiation Case groups included were pregnancy loss, low birthweight and stillbirth. Sample is thus not representative to whole population. Exposure assessed by telephone interview.
Guberan, Campana et al (1994)	Switz.	1781 women	Cross-sectional	Occupational exposure to electro-magnetic fields: Physiotherapists	= Large sample though self-administered questionnaire likely inaccurate for exposure measurement.
Muburak (1996)	Iraq	18 men 62 births	Cross-sectional	Occupational exposure to electro-magnetic fields: Power station workers	+ F / M - 18 power station workers Very small sample with no discussion of confounding factors.
Sadaat (2005)	Iran	51 men 110 births	Cross-sectional	Occupational exposure to electro-magnetic fields: Power linesmen	= Small sample with no exposure assessment. No significant differences in sex ratio between cross-sectional and control group.

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Teitelbaum and Mantel (1971)	U.S.A.	59,843 pregnancies	Ecological	Socio-economic status: Family Income and Education	+ M / F – Sex ratio increases from low to moderate rise in SES. Use of uni-dimensional measures of SES.
Ellis and Bonin (2002)	U.S.A. Canada	11,000 students	Ecological	Socio-economic status: 6 different SES measures inc. education, occupation.	= No associations between socio-economic status and sex ratio. Diverse socio-economic measurements used.
Catalano (2003)	Germany	2.3 million births	Time-series	Population stress: Economic decline	+ M / F – For East Germany in 1991 during a period of economic decline. Time-series modelling applied on national sex ratio trends (West and East Germany). No confounders analysed.
Catalano and Bruckner (2005)	Sweden	National births 1862-2004 (approx 11 million)	Time-series	Economic stress and living standards: Total private consumption of goods and services	+ M / F – Significant association between annual Swedish sex ratio and consumption of goods and services Well designed parsimonious study with use of Box-Jenkins modelling to identify auto-correlation in time-series. Decline in male births also identified in 1930s economic depression
Johns (2004)	England	272 women	Cross-sectional	Perception of individual health and living environment. Subjective Life Expectancy (SLE)	+ M / F- Small sample and no adjustments for confounders. Longer SLE predicted male birth amongst women interviewed (sig. association $p<0.05$)
Cameron and Dalerum (2009)	U.S.A.	399 billionaires	Case-control	Socio-economic status: Billionaire Wealth	+ M / F- Born to billionaires ($p<0.01$). Problems for data validity (whether all paternal sons and daughters recorded) and sex selective abortion of female foetuses in Asian countries not accounted for.

Mealey and Mackey (1990)	U.S.A.	1,314 women	Retrospective cohort	Husband's community status (Mormon church rank) and wealth. Polygny and paternal age.	+ M / F- For women married to men with highest community status Large though unrepresentative cohort (historical, strict religious group). Very small sample of highest status group (83 births).
Chacon-Puignau and Jaffe (1996)	Venezuela	577,196 births	Ecological	Socio-economic status (SES)	= Step-wise regression on multiple variables and sex ratio, including educational and occupational groups, marital status and age of mother and father. Multiple significant correlations shown and thus demonstrate contradictory findings for link with SES and sex ratio.
Magnuson and Bodin (2007)	Sweden	Approx 5.5 million births	Ecological	Socio-economic status: Father's occupation	+ M / F - For agricultural owners/managers and office managers Robust ecological investigation though period of analysis (1940 to 1949) coincides with World War II rise in sex ratio, possibly skewing results.. Potential confounding factor is therefore occupational exposure.
Maconochie and Roman (1997)	Scotland	549,048 births	Ecological	Maternal and paternal social class, maternal age and maternal height	= Large population analysis from 1975 to 1988 for all of Scotland with reliable data. Conclusion - "No evidence to suggest that gender determination is anything other than a chance process."
Marleau and Saucier (2000)	Canada	385 women	Retrospective Cohort	Social Class and Anxiety/Depression Score	= Psychological study with relatively small sample. Significantly less male births with lower self esteem scores

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Grant and Yang (2003)	New Zealand	377 women	Retrospective Cohort	Women listed in professional biographies, ‘achieving women’	+ M / F - For women listed in professional biographies in New Zealand. Relatively small sample over 40 year period with many confounding factors possible and none analysed or discussed.
Norberg (2004)	U.S.A.	86,436 births	Ecological	Partnership Status	+ F / M – For couples *not* co-habiting. Adjustment for confounding factors though multi-collinearity likely to exist for partnership status and socio-economic variables
Riis (1999)	Israel	approx. 1.5 million births	Ecological	Observance of religious laws: Orthodox Jewish and Moslem communities	+ M / F – In areas with strong observance of Orthodox Judiasm. Hypothesis that sexual abstinence around menstruation linked. Area-based measurement, therefore ecological fallacy likely.
Fukuda, Fukuda et al. (2002)	Japan	5373 pregnancies	Cohort	Maternal and Paternal Smoking	+ M, F- For mothers and fathers smoked more than 20 cigarettes per day. Large cohort though selection bias likely and no adjustments for confounders
Heron and Ness (2004)	England	9048 pregnancies	Cohort	Maternal and Paternal Smoking	= Large cohort and representative sample from UK population. No association observed between parental smoking and sex ratio

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Mills, England et al. (2003)	Sweden	1,552,790 births	Ecological	Maternal Smoking	= For maternal smoking and sex ratio Data from Swedish Medical Registry, cross-tabulation of sex at birth with category of non, moderate and heavy smokers for mother.
Parazzini, Chatenoud et al (2005)	Italy	1962 women	Cohort	Maternal and Paternal Smoking	= Smoking reported by interview with mothers. No significant associations between parental smoking and sex ratio.
Beratis, Asimacopolou et al. (2008)	Greece	2108 births	Cross-sectional	Maternal Smoking	+ M / F – For women who smoked. Only smoking status of mother is analysed. No discussion on representativeness of sample.
Koshy, Depisheh et al. (2010)	England	8960 births	Retrospective Cohort	Maternal and Paternal Smoking	+ F / - M For parents who are both heavy smokers (p<0.001). Well designed study with both hospital and community based cohorts. Smoking = > female births when controlling for socio-economic status, alcohol exposure and body mass index.
Dickinson and Parker (1994)	England	268,109 births	Ecological	Alcohol and Lead	+ F / - M For fathers occupationally exposed to alcohol and lead Meta-analysis undertaken with this and two previous studies. Exposures designated only on the basis of job type. No adjustments for confounders.

Lyster, Lloyd et al (1987)	England and Wales	5459 births and 3504 births	Meta-analysis	Alcohol: Occupational exposure	+ F / - M For alcohol handlers, publicans and barmen. No confounders analysed and no discussion of. Data from 2 studies in 1931, 1980-1982.
Qazi and Masakawa (1976)	U.S.A.	64 births	Case study and Meta-Analysis	Fetal-Alcohol Syndrome	+ F / - M Very small sample of fetal alcohol syndrome patients whose mothers were chronic alcoholics (11 patients). 2 other published reports added to analysis.
Catalano and Bruckner (2005a)	Sweden	276 months of national births	Time-series analysis	Population Stress – Prescriptions of anti-depressants and anxiolytics	+F / - M Sig results for 1st and 2nd month of pregnancy. Robust Box-Jenkins/Dickey analyses to identify and model auto-correlation. No confounding factors examined
Fukuda, Fukuda et al (1998)	Japan	82,130 male birth and 77,155 female births	Time-series analysis	Population Stress - Earthquake	+ F / - M Straightforward calculation of sex ratio 9 months post earthquake. No confounders considered. Query over accurate birth registration during earthquake disaster
Catalano, Bruckner et al (2006)	U.S.A.	91 months of New York city births	Time-series analysis	Population Stress – Terrorist Attack	+ F / - M For 5th month post September 11 attack. Box-Jenkins auto-correlation identified. No significant changes for other months shown. No confounders.
Catalano and Bruckner (2005b)	U.S.A.	168 months of Californian births	Time-series analysis	Population Stress – Terrorist Attack	+ F / - M For 3rd month post September 11 attack. Box-Jenkins auto-correlation identified. No confounders and 3 Catalano time-series analyses on peri-natal stress are <u>not</u> commensurate (sex ratio changes for 1st, 3rd and 5th months.)

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Saadat (2008)	Iran	469 births	Case-control	Population Stress - Earthquake	+ F / - M 11 months post Bam earthquake, Kerman region the control population. No confounders analysed or discussed. Query over accurate birth registration during earthquake disaster.
Hansen, Moller et al (1999)	Denmark	3072 pregnancies	Retrospective Cohort	Psychological Stress – Severe life events (Death or major illness/injury to family member)	+ F / - M Uses Danish comprehensive medical registry data-set with data linkage. No confounders included though severe life events likely to be spread across other population categories.
Ruckstuhl, Colijn et al (2010)	U.K.	16,834 births	Retrospective Cohort	Psychological Stress – Maternal job type	+ F / - M For ‘high psychological stress’ jobs. Job type category (multiple occupations grouped) is imprecise measure of occupational stress. Adjustment for confounders included
Obel, Henriksen et al. (2007)	Denmark	8719 women	Retrospective Cohort	Psychological Stress	+ F / - M For increased General Health Questionnaire score i.e. elevated psychological stress. Very large cohort with simple stratification of cohort, no adjustment for confounders.
Zorn, Sucur et al. (2002)	Slovenia	4966 and 1565 births	Time-series	Population Stress : Short Warfare Period	+ F / - M Straightforward calculation of sex ratio for Slovenia and Ljubliana 6 to 9 months post short war (26 June – 7 July, 1991)

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Khashan, Mortensen et al. (2009)	Denmark	1,349,099 births	Ecological	Psychological Stress – Death or serious illness to older children or partner	= Replication of Hansen et al. study using population based medical registry. Only article to refute the stress hypothesis.
Gibson and Mace (2003)	Ethiopia	324 births	Cross-sectional	Resource availability (Trivers-Willard hypothesis): Nutritional status	+ M / - F For higher nutritional status with anthropometric measurement of arm circumference area. Small study and no confounders analysed.
Stein, Barrett et al (2004)	Ethiopia	15,367	Cross-sectional	Maternal under-nutrition	= Replication of Gibson study with much larger sample across Ethiopia. No discussion on reliability of data that was sourced from national survey in developing country.
Stein, Zybert et al. (2004)	Netherlands	4250 births over 18 months (1944 to 1946)	Time-series	Maternal nutrition: Famine exposure	= Exposure based on 3 birthing institutions in Western Netherlands, an area of famine. Questions over reliability of data given wartime. Age adjusted sex ratios.
Mathews, Johnson et al. (2008)	England	740 women	Cohort	Maternal nutrition: Pre-conceptional energy intake	+ M / - F For highest third of pre-conceptional energy intake. Well designed study with detailed food diaries, stratified random sampling and confounders analysed.
Cagnacci, Renzi et al. (2004)	Italy	10,239 births	Retrospective Cohort	Body weight and Weight gain	+ F / - M For low pre-pregnancy weight. Representativeness of cohort questionable and pre-pregnancy weight recorded from participant recall.

Smits, de Bie et al. (2005)	Netherlands	5283 women	Cohort	Fertility: Time to Pregnancy	+ M / - F Greater probability of male birth for time to pregnancy > 12 months. Adjustment for large no. of confounding factors included.
Joffe, Bennett et al. (2007)	England, Denmark	49,506 pregnancies	Ecological	Fertility: Time to Pregnancy	= Well designed study with analysis of four large population surveys. No discernible trend for separate datasets or combination of all.
Barber (2004)	Global	Sex ratios from 148 countries	Ecological	Fertility – Fertility rates	+ F / M – For increases in fertility and thus decreased coital rate. Study compromised by data inaccuracy from developing countries (30% births not recorded)
Crawford and Davies (1973)	England	1459 births	Retrospective Cohort	Pre-pregnancy oral contraception	= Relatively small sample size with over-representation of higher socio-economic status participants
Rothman and Liess (1976)	U.S.A.	6109 births	Retrospective Cohort	Oral contraceptive use	= Contraception use established from questionnaire. Stratification based on total months of use.
Vessey, Meisler et al. (1979)	England	5,700 pregnancies	Cohort	Oral contraceptive use	= Biased sample, mothers aged 25 to 29. Replicates other findings on oral contraceptive use
Klinger and Glasser (1981)	U.S.A.	2986 births	Retrospective Cohort	Oral contraceptive use	= Maternal contraceptive use extrapolated from medical records. Simple sex ratio calculations, no confounders.
Møller (1998)	Denmark	514 men	Case-control	Testicular Cancer	+ F / - M Straightforward calculation of sex ratio amongst testicular cancer cases (252 boys and 288 girls) Control was expected sex ratio value from population i.e. 0.514

Author (Year)	Country	Sample size	Study Design	Exposure of interest	Main findings
Jacobsen, Bostolfe et al. (2004)	Denmark	3530 men	Case-control	Testicular Cancer	+ F / - M Stratification according to years pre and post diagnosis, and seminoma and non-seminoma. Significance for all cases and pre-diagnosis (> -2 years) No adjustments for confounders.
Moran, Novitski et al. (1969)	U.S.A.	2 million births	Ecological: Multivariate Regression	Paternal age	+ F / - M One of few early studies focusing on paternal age
Garfinkel and Selvin (1976)	U.S.A.	1.4 million births	Ecological: Multivariate Regression	Maternal age, Paternal age, Birth Order	+ F / - M For advancing parental age and birth order. Explains only 1% of the variation in sex ratio. Quadratic model applied to New York State data, 7 age categories.
Ruder (1985)	U.S.A.	1.67 million births	Ecological: Multivariate Regression	Parental ages, Birth order	+ F / - M For advancing paternal age and birth order (highest correlation co-efficient) Linear regression and logistic analysis performed on ungrouped data (uses mean ages at birth)
Ulizzi and Zonta (1995)	Italy	Approx 10.5 million births	Ecological: Multivariate Regression	Maternal age, Birth order, Firstborn proportion	+ F / -M For increasing maternal age. Quadratic function of maternal and firstborn proportion greatest predictor of sex ratio. Large samples from Italian population though where national sex ratio increases post-war.

Jacobsen, Moller et al. (1999)	Denmark	815,891 births	Ecological: Multivariate Regression	Paternal age, Maternal age, Birth order	+ F / -M For advancing paternal age and increasing number of children per plural birth. Logistic regression applied with categories of maternal and paternal ages (5 year intervals)
Nicolich, Huebner et al. (2000)	U.S.A.	62 million births	Ecological: Multivariate Regression	Maternal age, Paternal age, Birth weight	+ F / -M For both parents and increasing number of low weight births. Greatest co-efficient for father's age. Largest sample of any sex ratio investigation. Logistic regression model. No confounders analysed
Pollard (1969)	Australia	Approx 3 million births	Ecological: Linear Regression	Paternal age, Maternal age	+ F / - M For both paternal and maternal age. Correlation co-efficient very small. No multi-regression models shown in the article.
Rostron and James (1977)	Scotland	1,069,595 births	Ecological: Linear Regression	Maternal age, Social class, parity	= Analysis of Variance study. Multiple models of interactions .e.g. paternal age and social class
James and Rostron (1985)	England and Wales	All births from 1968 to 1977 in England and Wales	Ecological: Multivariate Regression	Paternal age, Maternal age, Birth order	+ F / - M For all factors both combined and independently. Still births also included in the analysis, therefore does not constitute the secondary sex ratio.
Guiterrez-Adan, Pintado et al. (2000)	Spain	All births from 1946 and 1981 in Spain	Ecological: Multivariate Regression	Maternal age, marriage age, age difference between husband and wife	+ F / - M For age of mother and decline in mean marriage age. Pearsons Correlation co-efficient, no analysis or discussion of confounders

Cann and Cavallis (1968)	Italy	888,154	Ecological: Multivariate Regression	Maternal age, Birth order	= Chi-square testing of nonlinear variation and linear regression. Only for the year of 1960.
Erickson (1976)	U.S.A.	5,349,446	Ecological: Multivariate Regression	Paternal age, Maternal age, Birth order	= Different method: Mantel-Haenszel approach and weighted linear regression. Sample of half U.S. births 1969 to 1972.
Imaizumi and Murata (1979)	Japan	3,734,057	Ecological: Multivariate Regression	Paternal age, Maternal age, Birth order	= Japanese births from 1973 to 1976. Weighted least squares methods used
Curtsinger, Ito et al. (1983)	Japan	Approx 5,000 parents	Ecological Univariate Regression	Maternal age, Paternal age, Birth order and Sequences of sexes at birth	= ‘Vertical Analysis’, much smaller sample than other parental age studies
Tremblay, Vezina et al. (2003)	Quebec, Canada	20,000 births	Ecological: Univariate,	Parental age, Birth order	= Investigation of unusually high sex ratio in province 1855 to 1899 (109 male births to 100 female births). No correlations with age and 20th Century decline.
Matsuo, Ushioda et al. (2009)	Japan	3,049 births	Retrospective Cohort	Parental age	+ F / - M For synergistic effect of both parent’s ages. Well designed study with representativeness of sample and confounders analysed
Ein-Mor, Mankuta et al. (2010)	Israel	35,837	Retrospective Cohort	Maternal age, Paternal age	= Systemic bias, no discussion of population context.

2.3.1 *Environmental Factors*

The ‘environmental’ results of the search include studies on 4 particular chemicals, a group of chemicals (pesticides), all of which have been identified as endocrine disrupting compounds. Research on nuclear radiation and electro-magnetic radiation was also selected and reviewed. Further environmental studies were grouped according to single or multiple pollution sources; specifically air pollution, and potentially combined effects including land and water contamination as well as poor air quality.

2.3.1.1 Dioxins

Dioxins contain some of the most toxic substances to humans, such as TCDD, produced by industrial activity, with common sources being PVC and wood burning, waste incineration and manufacture of pesticides.

The research on dioxins and male birth proportions include 6 analyses of cohorts. The biological hypotheses on mechanisms involved are not well developed in any of the research papers, with the chemical’s anti-oestrogenic properties only briefly commented on. The case for alteration of sex ratio from dioxin exposure relies predominantly on evidence from a large hazardous event 35 years ago in northern Italy.

The Seveso industrial accident, at a herbicides manufacturing plant in 1976, released up to 16kg of TCDD dioxin from 2,4,5-trichlorophenol and exposed up to 17,000 people in an adjacent town to 3kg of dioxin. Cohorts were recruited following the event and have been extensively examined in environmental epidemiological study with 2 such studies investigating sex ratio changes as a potential effect. The first study showed a dramatic reduction in secondary sex ratio 9 months after the release amongst both parents that were living in the most contaminated zone (Mocarelli, Brambilla et al. 1996). The second more comprehensive study found that paternal exposure to TCDD, as measured in serum samples collected at the time of exposure, were associated with a lower proportion of male births (Mocarelli, Gerthoux et al. 2000). In particular, the study distinguished that the offspring of men exposed before 19 years of age were particularly vulnerable to sex ratio decline and thus reproductive effects from dioxin. The findings from the Seveso research are validated by the accurate, timely measurement of dioxin concentrations which were incorporated into a follow-up study from birth data analysis. Frequently, other studies on alterations of male birth proportion post-hazardous events rely *solely* on an analysis of simple sex ratio time-series trends, which tend to include assumptions made on exposures rather than reliable biological measurement.

A further 4 cohort studies report no change, or slight rise, in sex ratio from exposure to dioxin-like compounds such as rice oil and Agent Orange (Michalek, Rahe et al. 1998; Rogan, Gladen et al. 1999; Schnorr, Lawson et al. 2001; Yoshimura, Kaneko et al. 2001). Significantly, the rice oil studies do not include exposure measurement and one Agent Orange investigation uses only *estimated* exposure measurement, based on hepatic elimination rates and dates of employment. However, the difference in sex ratio results from the Seveso and Agent Orange studies could be attributed to exposure from acute events vis-a-vis chronic occupational toxicity. This differing evidence may also reflect a dose-response effect from dioxin for sex ratio deviation as well as according the timing of exposure from the chemical.

2.3.1.2 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) are highly persistent and bio-accumulate in the environment owing to their lack of solubility in water. Their manufacture is now severely restricted having previously been widely used for electrical equipment and as plasticizers as well as being applied to fire retardants. The biological mechanisms of PCBs and sex ratio alteration is also not explored in depth, though reference is made to anti-androgenic and anti-oestrogenic properties which have been demonstrated in some animal experiments.

The 6 studies on biphenyls reviewed, each revealed associations between exposure and both upward and downward changes to secondary sex ratio. 4 of these studies, conducted in the U.S., contain robust methodology, with adjustments made for confounding factors, accurate measurements of exposure and consideration of potential biases. A retrospective cohort study in San Francisco found a 33% decrease in the chances of a male birth between women with the lowest and highest concentrations of PCBs (Hertz-Picciotto, Jusko et al. 2008). Serum specimens were collected from pregnant women in the 1960s when PCBs were ubiquitous industrial chemicals and exposure most likely. A similar study on accidental exposure to PBBs from animal feed in Michigan in 1973-74, analysed a cohort which had increased odds of male births from combined parental exposure to PBBs and PCBs (Terrell, Berzen et al. 2009). Furthermore, a cohort recruited in the Great Lakes region, was used in 2 studies of PCB exposure from consumption of locally caught fish, produces contrasting results for maternal and paternal exposure (Karmaus, Huang et al. 2002; Weisskopf, Anderson et al. 2004). Caution is exercised though as the cohort size used for both studies is much smaller making differentials between PCB levels, and sex ratio calculations, particularly crude. An Australian study, with similar robust methods, measures PCB content in breast milk of 850 pregnant women and found no significant change in male birth proportion eventuating (Khanjani and Sim 2007).

A study in the Russian Arctic, where indigenous people are particularly vulnerable to PCB hazard because of their traditional fish and whale diet, is also significant (International POPs Elimination Project 2006; Bjerregaard 2008). This reproductive health study, investigating other reproductive and birth indicators, revealed higher PCB levels equated with a lower offspring ratio whereas low levels of PCB led to an increased ratio (International POPs Elimination Project 2006). Potential bias and confounding factors were not discussed in the report, although associations with other reproductive indicators such as low weight births, and the stable population investigated, suggest such results are important. Overall therefore, associations between PCB exposure and sex ratio changes are variable though environmental health evidence suggests such reproductive effects are likely.

2.3.1.3 Pesticides

Some pesticides such as the insecticide DDT are organochlorine compounds which were first used to control lice and mosquitoes in the Second World War and then used extensively in post-war agriculture, particularly in the United States. The eventual banning of products such as DDT by the US government in the 1950s was one of the first legislative acts on environmental regulation. DDT, however, is highly persistent in the environment owing to its molecular structure and other pesticides are also still applied in current agricultural practice.

Biological mechanisms are not discussed substantially in the studies, though it is noted that some of the endocrine disruptive characteristics of such chemical types, which also includes PCBs, have been demonstrated in previous laboratory experiments.

The selected studies examining pesticides vary in type and include 1 case-control, 2 cross-sectional, 4 occupational-cohorts and 3 ecological investigations.

The case-control study concerns the skin disease of porphyria which occurred in south-eastern Turkey in the 1950s, as a result of exposure to hexachlorobenzene (HCB). The study reports a decline in the proportion of male births to severely-exposed porphyric women, although the statistical relationship is weak (Jarrell, Gocmen et al. 2002). Two Australian cross-sectional studies measured DDT and HCB levels in breast milk as part of the same recruited PCB group discussed above and results were also replicated with no significant changes in the resultant sex ratio (Khanjani and Sim 2006; Khanjani and Sim 2006).

The occupational cohort studies include two comprehensive epidemiological investigations which include serum collections and adjustment for confounders. Results are inconsistent, with Minnesota pesticide applicators displaying a male predominance at birth with exposure to herbicides and a reversed pattern for fungicide exposure (Garry, Harkins et al. 2002). In the second study, pesticide producers in Russia exposed to dioxin were associated with a decreased sex ratio, particularly amongst fathers (Ryan, Amirova et al. 2002). For the other studies, one is an investigation of greenhouse workers which yields no significant associations from a very large cohort and the second examines 30 workers exposed to DBCP (Bretveld, Hooiveld et al. 2008). A highly significant link is made in the DBCP study between impaired spermatogenesis which is accurately measured from sperm samples, exposure to DBCP, and a preponderance of 12 female to 2 male births (Potashnik, Goldsmith et al. 1984). Although the sample size is very low in the DBCP study, significant bio-markers are measured, and the unique combined evidence of biological mechanisms and sex ratio adjustment is significant.

The 3 ecological studies expand the research on pesticides to include geographical analysis, though only one study, from Brazil, reports a statistically significant decline in male births (Gibson and Koifman 2008). No confounding factors however were considered and the designation of polluted zones based solely on agricultural toxic sales is a crude method for assessing exposure. In the other two studies, more detailed assessments of exposure were undertaken, with measurement of water quality and multiple databases on chemical use (Whorton, Wong et al. 1989; Clementi, Causin et al. 2007). These produced no results showing any associations with changes in male birth proportion. Evidence of alterations in sex ratio in populations as a result of pesticide exposure is reasonably compared with dioxins and PCB studies. A tentative hypothesis of a pre-dominantly male route to exposure and effects on sex ratio is partly sustained through the Russian occupational study on pesticide workers and the DBCP research findings.

2.3.1.4 Mercury

One research article identified which examines mercury pollution is a retrospective ecological study on methyl-mercury poisoning in Minimata City, Japan (Sakamoto, Nakano et al. 2001) . The analysis shows a significant decline in male birth proportion between 1955 and 1959 when the pollution was most severe as well as a rise in male stillborn foetuses during this period. Although the results, from a large sample of 20,487 births, suggest a possible link to maternal and teratogenic contamination, there is still a possibility of ecological fallacy given the lack of measured exposure and discussion of confounders. A more detailed follow-up study, similar to Seveso, has not been completed, possibly because early recruitment of an exposed cohort at the time of the pollution event did not similarly occur.

2.3.1.5 Lead

Lead, a ubiquitous pollutant from petrol, paint, water pipes and electrical batteries, is still prevalent in the environment despite recent regulation by governments. One comprehensive study of a cohort in Mexico, measured lead levels from spectroscopy of maternal and umbilical cord blood and X-rays of the patella and tibia. Results showed no association was found between maternal and foetal lead exposure, and secondary sex ratio (Jarrell, Weisskopf et al. 2006). This study uses accurate exposure measurement and would suggest no link with lead and altered sex ratios, however, long term low level exposure of lead to populations, in combination with other endocrine disrupting compounds, has as yet not been explored.

2.3.1.6 Boron

Boron is a naturally occurring trace element in plants rather than a man-made product released into the environment. At higher doses it can be toxic to humans and some studies have identified possible reproductive effects on borate workers and their partners (Whorton et al. 1994). One study selected, establishes a sex ratio decline in a boron exposed village from drinking water, in contrast to no such alteration in a nearby control locality (Sayli, Tüccar, & Elhan 1998). This simple case-study of sex ratios in different areas however, is small in scope and subject to ecological fallacy.

2.3.1.7 Local Exposure: Multiple Media

5 sex ratio studies focus on localities which have high concentrations of air pollution and/or land and water contamination. Three of these studies are straightforward case-studies showing annual sex ratio figures and do not contain exposure measurement or analysis of multiple variables (Fertmann, Schumann et al. 1997; Kozlov 1999; Liu, Li et al. 2010). Levels of significance are calculated against control populations and the 3 studies do demonstrate significant skewing of male birth proportion, both downwards and upwards. A Canadian study has received considerable media attention owing to the extent of sex ratio decline affecting a First Nation community (Mackenzie, Lockridge et al. 2005). Although no confounding factors are examined in this study, a community based health assessment currently being undertaken could provide a model on how to develop follow up studies on such heavily polluted localities. Such assessments are therefore required before conclusions can be derived on linking identifiable local areas adjacent to pollution with sex ratio alteration.

An important study also identified from the search is a geo-statistical analysis on a large population of regional France (Viel, Floret et al. 2005). Spatial statistical scanning was adopted to detect clusters of low sex ratio and then assess their proximity to solid waste incinerators or areas of intense agricultural activity. No results suggested links to sex ratio from endocrine disruptor pollution, although the level of dioxin emissions is not established and only a single spatial level, by ‘cantons’, is used in the study.

2.3.1.8 Air Pollution

Research exploring air pollution and its possible influence on secondary sex ratio already include dioxin emissions in Seveso and the Local Exposure cited studies. However, there are 7 studies which exclusively examine air pollution, 3 of which involve geographical analysis, 2 are ecological studies, and 2 are case studies.

The 3 geo-analytical studies are conducted in central Scotland and display all possible outcomes: no change, and increase and decrease in sex ratio. The study showing upward skewing investigates pollution from a steel foundry adjacent to a small town, and includes robust measures of exposure with soil sampling and plume analysis (Lloyd, Lloyd et al. 1984). In a follow-up report, the inclusion of a co-indicator for health impact, the incidence of respiratory cancer, and its corresponding rise over the same time period, further strengthens the research findings (Lloyd, Smith et al. 1985). However, although significance is established, the small number of births analysed (n = 95,105,111) in the three time periods may limit the importance which can be attributed to such results.

The other two studies were undertaken in a neighbouring area and use similar environmental measurements. In the earlier study particular districts were designated ‘at risk’ areas based on their proximity to two waste incinerator plants, the prevailing wind directions, reports from local residents about fumes and the identification of polycyclic hydrocarbons in surface soils (Williams, Lawson et al. 1992). One postcode area was considered to be the most at risk area from airborne pollution and this locality also displayed the lowest sex ratio. The follow-up study compared sex ratios of births and mortality in 12 Scottish localities with residential exposure to pollution and 12 localities with negligible airborne pollution (Williams, Ogston et al. 1995). These results indicated, however, that whereas lung cancer mortality was higher amongst the exposed localities there was no association between the exposed areas and altered sex ratios. The weaknesses for such research, which is stated by the authors, is the ‘deductive guesswork’ involved in assessing exposure, partly because there was no formal monitoring network of air pollution. These investigations though are highly relevant to this study, given not only the pioneering nature of such work in birth sex ratios as a potential indicator for environmental health effects, but also its local context in Central Scotland. Environmental monitoring resources are now available, in the form of SEPA’s Scottish Pollutant Release Inventory and Falkirk Council’s Air Quality Management Strategy which incorporate pollutant concentration recording.

The ecological study undertaken in Sao Paolo, Brazil uses data from air pollution monitoring stations. The limitation of the study however is that concentrations of pollution are only associated with the exact period of live-births rather than timing of conceptions 9 months previously or early pregnancy (Lichtenfels, Gomes et al. 2007). Temporal analysis is also not given in a study on air pollution from a petroleum plant in

Taiwan and a similar study finds no associations with abnormal sex ratios (Yang, Cheng et al. 2000; Yang, Tsai et al. 2000).

2.3.1.9 Nuclear Radiation

Research focused exclusively on nuclear radiation includes 1 time-series analysis, 3 ecological studies with environmental measurement and mapping, and 2 occupational studies. These investigations concern radioactivity in the form of air pollution from nuclear accidents and testing, and workers' exposures in the nuclear industry. The primary mechanisms speculated as responsible for sex ratio alteration concerns the higher vulnerability to male foetuses from environmental stresses as well as a detailed explanation of X-chromosome linked dominant mutations in irradiated fathers.

The Chernobyl nuclear accident in 1986, synonymous with radioactive pollution, is investigated in 3 studies, with mixed results. A sex ratio anomaly is highlighted in the Czech Republic with newborn girls exceeding newborn boys in November 1986, 3 months after the accident, compared to 599 other months in the last 50 years where sex ratio maintained the usual slight male excess (Peterka, Peterkova et al. 2004). A follow-up study produces regional Czech sex ratio trends and assesses exposure using measurements of radionuclide's deposits and rainfall intensity to produce maps (Peterka, Peterkova et al. 2007). The results show an association with lower proportions of male births in eastern regions where radioactive exposure is greatest. It is suggested that radioiodine in the fetal thyroid glands and consequent death of male fetuses is the mechanism for the reduction in male live births; however medical data shows no increase in spontaneous abortions during the November 1986 period. In contrast, a

German study finds a positive association of increasing odds of male birth between 1986 and 1991 and radioactive exposure in Bavaria, the former GDR, and West Berlin (Scherb and Voigt 2007). Details though, on measurement of ecological exposure from different regional environmental agencies are not clearly established in the paper and likely to be inconsistent.

More robust measurements of radiation exposure are stated in a study of nuclear testing in Kazakhstan where soil samples and meteorological conditions were obtained and mathematical modelling used (Mudie, Gusev et al. 2007). Findings from this research did not establish any significant association between sex ratio and parental radiation exposure.

The occupational research includes a large survey of 38,000 U.K. Nuclear employees and one much smaller; of men employed at the Sellafield nuclear plant in Cumbria, England (Dickinson, Parker et al. 1996; Maconochie, Roman et al. 2001). No evidence of sex ratio adjustment was found in either of these studies though exposures are only measured by length of service rather than biometric assessment.

2.3.1.10 Electro-Magnetic Fields

Alteration in birth sex ratios have been suggested for both high and low frequency electro-magnetic fields. Experiments on animals and in-vitro have demonstrated abnormal reproductive effects from exposures to such powerful fields. For male reproductive health, possible effects of radiofrequency radiation shown in epidemiological studies include reduced sperm motility and concentration, and potential lower survival of spermatocytes in the womb. Birth sex ratio investigations include 6 analyses of retrospective cohorts, one cross-sectional and one ecological study. All of these papers involve assessment of occupational exposure; including physiotherapists, Navy submariners, electric sub-station workers and power linesmen.

A reliable Norwegian Navy study, which reports a lower proportion of male births amongst higher exposed employees, utilises a comprehensive survey on self-reported exposure and controls for confounding factors such as smoking and age (Baste, Riise et al. 2008). Unusually for such type of research, the numbers examined are high, with 10,497 respondents in the survey. This partly explains statistical significance being established for high exposures and low sex ratios, such as 0.476 to 0.488 for birth proportion and number of births (N) being 1457 and 2158. Such large numbers for an occupational cohort study, together with similar associations for infertility in the investigation, further validates the findings.

The ecological study makes use of Norway's comprehensive medical birth registry and tabulates sex ratios per parent's employment group for all births from 1970 to 1993 (Irgens, Kruger et al. 1997). The male proportion of births was slightly reduced for men in electro-magnetic field industries, such as aluminium, iron and manganese works, whilst for women the ratio was significantly lower. Errors are likely to have occurred from misclassification of occupation categories and the levels of exposure, potentially modelled by employment duration and/or specific job within the industry, cannot be estimated. The results suggest strongly however, the female reproductive health impact of a reduction in male offspring births from prolonged exposure to low-level electro-magnetic radiation. There is also the potential, gleaned from this research, for exploring combined impacts of lead and poly-cyclic hydrocarbons (PAHs) in such industries. The female reproductive effect is further emphasised by a study on Danish physiotherapists where a statistically significant reduction in the male proportion of births is also reported (Larsen, Olsen et al. 1991). It requires stating though that there was not a significant difference in spontaneous abortions occurring between highly exposed and un-exposed females.

Two cohort studies do not replicate these findings where analysis is conducted for both male and female physiotherapists (Knave, Gamberale et al. 1979; Guberan, Campana et al. 1994). Sex ratios for small samples of workers exposed to electro-magnetic fields are calculated in 2 letters to journals, with one result displaying highly significant downward skewing though no confounders are considered (Mubarak 1996; Saadat 2005).

2.3.2 Socio-Economic and Demographic Factors

Studies categorised as socio-economic and demographic are from the social science disciplines and principally concern socio-economic status, parental age and behavioural and socio-cultural constructs which are not health related. It was important to distinguish between sociological, psychological and cultural factors investigated which were not generally attached to health behaviour, as the methods for such studies tended to be more social science, rather than epidemiologically, based.

2.3.2.1 Social-Economic Status

Twelve studies were identified which primarily analysed social-economic status and male birth proportion, whilst 6 other studies included this as a secondary factor for investigation. These included 7 ecological and 3 cohort studies and 1 retrospective case-control study and 1 study of biographies.

This particular research pre-dominantly concerns testing of the Trivers-Willard hypothesis which claims that an adaptive variation in sex ratio, based on natural selection, can be attributed to parents aiming to maximise reproductive success. The hypothesis predicts that parents in better condition and with improved living environments are more likely to have male offspring, as sons will eventually produce more offspring than daughters. One study pre-dates the hypothesis but is frequently used as evidence for the ensuing Trivers-Willard debate (Teitelbaum and Mantel 1971; Ellis and Bonin 2002). A further subject discussed in 2 studies is periodic declines in male birth proportion in countries as a result of contractions in national economies (Catalano 2003; Catalano and Bruckner 2005).

In testing the Trivers-Willard hypothesis, four of the analyses on population data (ecological) contained results which supported the proposition. With scant analysis of confounding factors in each study, the results though are subject to 'ecological fallacy'. The case-control and biographies studies offer similar supportive results. However, both samples used are low and contain bias (Johns 2004; Cameron and Dalerum 2009). Meanwhile, sex ratio calculations from a birth cohort of nineteenth-century Mormons show an inconsistent pattern rather than support for the Trivers-Willard attributed by the authors (Mealey and Mackey 1990). A similar discrepancy in results is shown in a Venezuelan study where educational status correlates with declining sex ratio whereas occupational level corresponds with a slight rise in male birth proportion (ChaconPuignau and Jaffe 1996). There are problems in interpreting findings from the use of single variables which are used as measurements of economic disadvantage. This is because low socio-economic status, or poverty, is now conceptualised as an outcome from a multitude of factors e.g. income, access to services, employment, schooling.

An analysis of individual data on fathers' occupation in Sweden between 1940 and 1949, using 523,671 population registers, presents correlations between higher status occupations and increased male birth proportion (Magnuson, Bodin et al. 2007). Such findings however are qualified by unremarkable statistical significance in some occupation categories (p=0.022), and that differences could be attributed to *occupational exposures*, as opposed to a strategic reproductive form of natural selection.

An earlier robust multi-variate demographic study in Scotland and two well designed large retrospective cohort studies refute the hypothesis (Friedlander and Benmoshe 1986; Maconochie and Roman 1997; Marleau and Saucier 2000). Results for socio-economic status influencing sex ratio are therefore inconsistent and methodological limitations exist for confirmative studies. However, such contradictory findings may reflect the variations in measurement of social-economic status between the studies and/or the statistical methods adopted.

2.3.2.2 Sociological or Psychological factors

There are 4 studies assessing sociological or psychological factors; including two ecological studies, a cross-sectional investigation and a case study of women's biographies.

The maternal dominance hypothesis, predicting that women with dominant personalities and greater testosterone levels are more likely to sire sons, is investigated in two of the studies. The proposition is advocated by a single investigator, Valerie Grant, who adopts an evolutionary psychological perspective to sex ratio study by reviewing multiple animal and human studies and proposing that, universally, females physiologically determine the sex of a baby through testosterone levels. In research conducted, analysis of 'high-achieving' women in New Zealand from written biographies reveals a greater propensity for male births, as well as childlessness and an unmarried status (Grant and Yang 2003). Such a straightforward uni-variate case-study is somewhat limited however and the hypothesis is potentially contradicted in that 'high-achieving' men, also analysed in the study, sired more daughters than sons. In the cross-sectional study, maternal personality questionnaires are reviewed from 3 groups together with corresponding sex ratios and a link of maternal dominance and male births is once again presented (Grant 1990). However, the analysis of only 85 women carries little statistical power and selection bias is inherent in the recruitment of at least one group.

A further gendered perspective is adopted in the ecological study of partnership status whereby a prediction is made, in assuming the Trivers-Willard hypothesis of sex allocation, that cohabiting partners are more likely to produce sons. Such a link is demonstrated with an analysis of five large population surveys totalling 86,436 births, where 49.9% of births were male amongst those who were not living with their partner (Norberg 2004). Although confounding variables are accounted for, the multiple regression analysis may be weakened by multi-collinearity as many other social and demographic factors can be correlated with partnership status.

In the second ecological study, a univariate and multivariate analysis is made of 1.5 million births in Israel with emphasis on sex ratio differences between Jews and Moslems (Riis 1999). It is speculated that the higher proportion of male births in orthodox Jewish communities may be attributed to strict observance of religious laws and abstinence of sexual intercourse during menstruation. This leads to coital rates occurring in the most fertile days of a woman’s cycle and the greater probability of a XX spermatozoa being fertilised owing to its greater motility. The timing of intercourse hypothesis is also linked to other demographic and health-related factors. Although the study includes some approximation, as sex ratios are calculated by area rather than individual cases, its unique, elucidating focus on socio-cultural determinants at reproduction is potentially instructive.

2.3.2.3 Parental Age

Searching for parental age as a factor influencing sex ratio presented 19 studies for review, 17 of which are ecological investigations, using either multiple or logistic regression, and 2 are analyses of cohorts.

It is posited parental age may lead to a lowering effect on male birth proportion owing to a number of possible mechanisms. Rationales include, hormonal changes in ageing males, such as lower androgen concentrations, and thus skewed ratio of X and Y bearing spermatozoa or less likelihood of Y bearing spermatozoa fertilising. There is also clinical evidence of DNA damage to spermatozoa from oxidative stress, with such damage likelier for advancing age. Less frequent coitus is also proposed for older partners with a consequently lower conception sex ratio because of the timing of conception hypothesis and less chance of an XX spermatozoa fertilising.

As expected, the ecological studies vary significantly in terms of size of populations, regression methods adopted, such as multiple, logistic and quadratic function, and control for other variables, such as socio-economic status and birth order. 12 of the 17 regression analyses establish models which predict a negative association between paternal age and sex ratio. These study sizes are large, ranging from 800,000 up to 62 million, and usually include all recorded births within countries over periods of years, sometimes decades (Moran, Novitski et al. 1969; Garfinkel and Selvin 1976; Ruder 1985; Ulizzi and Zonta 1995; Jacobsen, Moller et al. 1999; Nicolich, Huebner et al. 2000). The largest sample size of births, in the U.S. and following part of the period of recent sex ratio decline (1964 to 1988), predicts a 28% decrease in the range of male birth proportion for Whites from observed increases in the fathers' age (Nicolich, Huebner et al. 2000). The combined influence of both parents age is also relatively strong in this and other studies, with maternal age being identified as a further predictor of declining sex ratio as population sizes get larger (Pollard 1969; Rostron and James 1977). There is also a geographical variation in the research whereby maternal age association is reflected in Spain and Italy whereas in the U.S., Australia, and England

and Wales, paternal age is the defining predictive variable (James and Rostron 1985; Ulizzi and Zonta 1995; Gutierrez-Adan, Pintado et al. 2000).

Of the five ecological studies producing the null hypothesis, a multi-factorial analysis on secondary sex ratio in the U.S. between 1969 and 1971 is the most significant as it is the only study with a comparable sample size of 5 million births (Cann and Cavallis.Ll 1968; Erickson 1976; Imaizumi and Murata 1979; Curtsinger, Ito et al. 1983; Tremblay, Vezina et al. 2003). The use of a different statistical technique, Mantel-Haenszel and weighted linear regression, may explain the lack of any significant correlations with parental age, in contradiction to previous predictive models of the same U.S. data. This technique does not assess for importance of factors in explaining variation, the correlation coefficient (X^2), except relying on its sensitivity to ordered trends in the variables. Therefore, given the correlation coefficients are reasonably low in the other studies it is not surprising that the Mantel-Haenszel method results are not predictive. Significantly, the study highlights that caution should be attached to findings from large population analyses as given that X^2s are usually less than 10%, 'there is still substantial variation in the 'ratio' which is 'unexplained' by the 'independent' variables.' .

The 2 cohort studies identified contain contradictory results. A Japanese study affirming the age hypothesis, is from a recruited cohort, has reasonable representativeness and includes examination of confounding factors (Matsuo, Ushioda et al. 2009). The alternative Israeli study shows unremarkable variation in sex ratio across age bands, and although it is a much larger retrospective cohort, the sample is subject to systemic bias from one hospital with no discussion given on population context (Ein-Mor, Mankuta et al. 2010)

2.4 Part 1 – Summary and Conclusion

This chapter presents Part 1 of the scoping review on the birth sex ratio subject, detailing methods used in a thorough literature review of relevant studies from three literature databases and examining environmental and social factors under investigation. The inter-disciplinary nature of the topic is demonstrated with a variety of methods conducted to analyse potential determinants and sex at birth outcome. Probable environmental influences derived from toxicology studies include acute levels of dioxins and long-term exposure to PCBs. More generic investigations of pollution across multiple media types, most commonly air emissions, suggest heavily exposed populations as vulnerable to birth sex ratio decline. Amongst demographic factors, advanced parental age can be highlighted as likely to influence the secondary sex ratio, according to consistent results over recent and older studies. Meanwhile, findings on socio-economic status as a deterministic variable, ostensibly testing the Trivers-Willard hypothesis, are inconsistent. This then completes the results of eligible studies of environmental and socio-economic/demographic factors and the review is continued in the next chapter by presenting results on health-related determinants.

Chapter 3: Potential Determinants of Birth Sex Ratio Change in European and North American populations: Scoping Review Part 2 – Health-related Determinants, Generative Reproductive Mechanisms, Synthesis and Discussion.

3.1 Introduction: Scoping Review - Part 2

The second part of this scoping review focuses on studies of health-related determinants emerging from the systematic searches as well as a small narrative review on related mechanisms and indicators relevant to the study. Synthesis of parts One and Two forms the discussion component of the overall scoping review. Finally, research questions emerging from this extensive scrutiny of sex ratio inquiry and related factors are stated.

3.2 Literature Review: Results - Health-Related Determinants

The broad category of health-related determinants focuses on epidemiological inquiry and study designs in health science, namely smoking, alcohol, diet, and medical interventions. Stress is also classified in this section of the review given its strong association in epidemiological research and link to various diseases.

3.2.1 Smoking

Sex ratio research examining smoking as a potential factor includes 2 cohort studies, 1 ecological study and 3 case-control investigations.

Smoking has long been investigated for effects on reproductive health. However a cohort of 5372 women attending 2 obstetric clinics in Japan was the first to indicate a possible link between heavy smoking and lower male births (Fukuda, Fukuda et al. 2002). The biological mechanisms involved however are not substantially discussed in this and subsequent research with only sperm motility weakened by tobacco pathogens described as a possible explanation.

The early Japanese research has been criticised for selection bias and confounding, and two important studies have since contradicted these findings. Firstly, there is an analysis of the Avon Longitudinal study in Bristol, which is a large cohort and is widely recognised as representative of the UK population (Heron and Ness 2004). Secondly, an ecological study includes analysis of 1,552,790 births in Sweden between 1983 and 1997, though significantly this only delineates between smoking and non-smoking women, not the 'dose' of smoking (Mills, England et al. 2003).

The 3 retrospective case-control studies include one in support and two in refutation of the smoking hypothesis (Parazzini, Chatenoud et al. 2005; Beratis, Asimacopoulou et al. 2008; Koshy, Delpisheh et al. 2010). A recent analysis and community survey of births in Liverpool includes controls for confounding factors, and contains results similar to the initial Japanese study (Koshy, Delpisheh et al. 2010). These investigations found skewed low sex ratios amongst parents who were *both* heavy smokers. Therefore, although evidence does not suggest an overall progressive decline in proportion of male births according to the dose of smoking pathogens, *heavy* parental smoking in particular population groups may be implicated. The Liverpool study does recognise though that environmental pollution may be a significant confounding factor which has not been accounted for in the results.

3.2.2 Alcohol

Three studies on alcohol and sex ratios resulted from the search; a meta-analysis, a clinical case report, and a cross-sectional occupational study. The meta-analysis only includes 3 studies, one of which is the occupational study identified in the search and the authors own analysis of job type data showing no variation in sex ratio (Dickinson and Parker 1994). The occupational cross-sectional study reveals a statistically significant lower male proportion of births for workers exposed to alcohol, however the route and degree of exposure is not discussed or measured (Lyster, Lloyd et al. 1987). The clinical study features sex ratios of patients with fetal alcohol syndrome and a significant excess of female births for the 64 cases (Qazi and Masakawa 1976). Such skewing may show possible teratogenic effects from alcoholism; however it is difficult to transpose such a hypothesis to a wider population where alcohol consumption is more moderate. In summary, there is insufficient evidence from research undertaken to assess whether alcohol, either consumed or through occupational exposure, has an effect on the male proportion of births in populations.

3.2.3 Stress

Evidence of a possible link between decreased male birth proportion and stress was initially suggested in 1974 when Lyster reported altered sex ratios post the 1952 'London smog' and 1965 flood in Brisbane. Biological hypotheses on psychological distress, human reproduction and the proportion of sexes at birth have since developed, and testing of these is implicit to the research conducted.

One explanation is reduction in the frequency of coitus amongst stressed populations, as this decreases the likelihood of conception in the early part of menstrual cycle and thus the probability of male fertilisation. There is also a suggestion that stress can reduce sperm motility which can also lower the sex ratio at conception. Population stressors affecting females could also lead to endocrinal changes which induce spontaneous abortions, disproportionately affecting weaker male fetuses. The Trivers-Willard hypothesis also contributes to such claims, whereby parents with a poor resource base are likely to be psychologically stressed and thus a natural selection mechanism occurs where female births are more likely to occur.

Of the 12 studies identified from the search, 11 contained results which supported the stress hypothesis and only 1 reported no association. Complex and simple time-series analysis was conducted in 6 of these investigations and 6 involved studies of cohorts, both recruited, and extrapolated from national databases.

Support for the stress hypothesis from time-series analysis rests on the key assumption that a particular event, such as a natural disaster or period of economic contraction, is the sole *cause* of a 'drop' in sex ratio. One study however does detect a statistical relationship between sex ratio in Sweden and doses of anti-depressants prescribed to women in the first trimester of pregnancy, a robust proxy for anxiety in the population (Catalano, Bruckner et al. 2005). In the remaining studies there are no analyses of confounding factors in any of the studies or measurements of exposure to stress, such as surveys of the affected populations (Fukuda, Fukuda et al. 1998; Catalano, Bruckner et al. 2005; Catalano, Bruckner et al. 2006; Kemkes 2006; Saadat 2008). Notwithstanding such limitations the significant falls in birth sex ratio in all five of these studies suggests further investigation for such events is warranted. Further, in the case of environmental hazards, which include Seveso and Minimata City referred to earlier, it is important to isolate whether pollution or stress, or the combination of both, is responsible for periodic declines in male birth proportion.

Of the 4 cohort studies identified which support the hypothesis, two are methodologically compromised and two contain significant findings. An analysis of national birth registry data does not account for confounders, and a further study uses somewhat questionable job stress categories as proxy indicators for stress (Hansen, Moller et al. 1999; Ruckstuhl, Colijn et al. 2010). A Danish study however, of 6643 pregnant women undertakes robust exposure measurement through the use of the General Health Questionnaire. Despite a degree of bias inherent in such self-administered questionnaires the method is reasonably reliable, and results show a decrease in male birth proportion with increasing *levels* of stress (Obel, Henriksen et al. 2007). An analysis of sex ratio trends in the city of Dubrovnik during the 10 day Slovenian war demonstrates not only the common pattern of lowered male births but also a concurrent finding of decreased sperm motility (Zorn, Sucur et al. 2002).

Although the sample of sperm measurements of 39 men is small, the inclusion of research on associated biological mechanisms is of significant value to knowledge on the stress hypothesis.

A follow-up study to the Danish survey, using this birth registry data, cross-references sex of birth with the occurrence of severe life events and extends the time period for investigation (Khashan, Mortensen et al. 2009). The results however, are broadly similar to the first study despite a claim of possible refutation of the stress hypothesis. There is though discussion of a synergistic effect of combined stressors, such as economic recession and severe life events, which resulted in lower sex ratio for a particular time period (1980 – 1992). Indeed, given the findings from 10 of the 11 studies, there is reasonable evidence that severe stress, potentially for multiple reasons, can alter the proportion of male births downwards in a given population.

3.2.4 Diet/ Nutrition

Research on sex ratio changes linked to diet covers a number of associated parameters, including calorific intake, weight gain, malnutrition and mean height and weight. 3 cohort studies and 2 ecological studies were identified with one being time-series based.

The rationale for such investigations is strongly associated with natural selection and the Trivers-Willard hypothesis. The theory promoted is that as male offspring in-utero have higher caloric demands, maternal reproductive 'strategy' is geared towards producing males during beneficial environmental cycles and females when environmental stressors are greatest. Scientists have also speculated that male births may be correlated with maternal diets of high fatty acid or oestrogen content with electrolyte balance in humans being pivotal to sex ratio adjustment, as is also demonstrated in some rodent experiments.

One cohort and anthropometric study on an agro-pastoralist community in Ethiopia found an association between malnutrition and lower sex ratios whilst ecological census research on a wider Ethiopian population contradicted these results (Gibson and Mace 2003; Stein, Barnett et al. 2004). Similar results were established for the Dutch Hunger Winter in 1944-45, a retrospective study using logistic regression finding no associations between recorded rations at that time and male proportion of births (Stein, Zybert et al. 2004). The discrepancy in results may be explained by the varied methods, with the anthropometric survey using more accurate measurement for nutritional status of tricep skinfold markers. However, it is questionable whether such research translates to modern Western populations where malnutrition during famines cannot accurately represent a comparable measurement of diet and nutrition.

A cohort study was also undertaken in U.K. which followed 740 women attending antenatal clinics and who also completed food frequency questionnaires (Mathews, Johnson et al. 2008). The major finding of the study is that high pre-conceptional energy intake is associated with increasing male birth and therefore the researchers conclude that dietary changes in Western populations could explain the overall declining sex ratio phenomenon. The use of food frequency questionnaires, recognised as a reliable and valid epidemiological method, and accounting for confounding factors of age, smoking and education, strengthen the conclusions that are offered from the research. The systematic searches however, revealed no confirmative studies for these findings. The final cohort study is retrospective and is conducted on maternal weight. A shortcoming of the investigation is that use of patient recall to measure weight is subject to inaccuracy (Cagnacci, Renzi et al. 2004).

3.2.5 Reproductive Health and Interventions

Research on factors specifically associated with reproductive health, including medical interventions, diagnoses and sexual behaviour, was also identified from the search. These included 7 cohort studies and 1 case-control study, 2 meta analyses and an ecological study.

It is speculated that the timing of conception can influence the sex of offspring during reproduction, as has already been discussed in relation to factors such as stress and religious laws. An extension of this hypothesis is that female cervical mucal viscosity can slow X bearing sperm and therefore fertilisation takes longer and male offspring is more likely. In this context, two cohort studies examine time to pregnancy and produce contradictory findings (Smits, de Bie et al. 2005; Joffe, Bennett et al. 2007). Both studies are robust, although the negative finding for sex ratio deviation uses four large European population surveys whereas the alternative positive study may be subject to bias as recruitment of the cohort is not clarified. With either result however, it is worth emphasising that the analyses of fertility are over long periods rather than specifically on the timing of intercourse, which is commented on in the previous studies relating to stress, parental age and societal ‘codes’.

The ecological study, of 148 countries, addresses total fertility and coital rate, as well as contraceptive use, and shows correlations with declining sex ratios (Barber 2004). The study however is inevitably compromised by data inaccuracy given that an estimated 30% of births in some developing countries remain unregistered (UNICEF 2007).

There are 4 cohort studies which examine the contraceptive pill and sex ratio, and all of these find no association (Crawford and Davies 1973; Rothman and Liess 1976; Vessey, Meisler et al. 1979; Klinger and Glasser 1981).This research was conducted at a time of introduction of the contraceptive pill and public concern over possible links to birth defects. Three of these studies use robust methods to demonstrate no impact on sex ratio or any deleterious effects. A final study produces similar negative results though systemic bias exists with recruitment of the cohort.

The meta-analyses concern artificial insemination and natural family planning, with both compiled data in the studies indicating a lower proportion of male births (Mortimer and Richardson 1982; Gray 1991). Fresh and cry-stored semen resulted in a reduced sex ratio in the U.K. for artificial insemination whilst coitus during the most fertile cycle led to a similar effect. This would seem to challenge the timing of intercourse hypothesis; however the largest study by far, of the 6 reviewed, is not sufficiently weighted in the meta-analysis.

As discussed in regard to eligibility criteria for the review, testicular cancer was deemed as significant as a medical factor for selection because of the suggested link to the environmental endocrine hypothesis and testicular dysgenesis syndrome (Skakkebaek, Rajpert-De Meyts et al. 2001). Two studies are identified which concern this disease, 1 case control study and 1 retrospective cohort study (Pera, Moller et al. 1998; Jacobsen, Bostofte et al. 2000). In the first study the cases of 514 men diagnosed with testicular cancer and their offspring's sex is recorded from 2 years up to their diagnosis. Significance is established in the difference between the case and control group, a lower ratio of 0.47 compared to 0.52. There is little detail in the paper though on the methods applied in the study, such as how sample obtained and case histories examined to negate possible confounding factors. The second study replicates these findings by analysing a large cohort obtained from the national Danish cancer registry. In a study population of 3530 men diagnosed with testicular cancer between 1945 and 1980, a significantly lower male proportion of births is displayed compared to Danish men overall. The utility of such studies are not necessarily solely linked to endocrine disruptors, particularly given the biological mechanisms for such associations are not developed. There is also an emerging characteristic of deteriorating male reproductive health being linked to a decline in birth sex ratio.

3.3 Reproductive Mechanisms and Linked Indicators – Narrative Review

Factors affecting the determination of the human secondary sex ratio and the factors or chemical agents responsible also merit some further review of reproductive and peri/post-natal epidemiological research. Biological mechanisms are described and occasionally theorised in many of the preceding studies from the literature review, the reproductive physiology and processes discussed but are not elaborated on by considering population health trends and data. Scanning of relevant research and literature also revealed that some connected mechanisms to secondary sex ratio alteration are not included in sex ratio studies e.g. sperm DNA fragmentation. A holistic view of the secondary sex ratio phenomenon, which includes a review of generative mechanisms, is instrumental to furthering knowledge of potential determinants. As secondary sex ratio is included in many broader reproductive health investigations or reviews it would also be requisite to relate any sex ratio study to other measures such as birth outcomes and semen characteristics. A brief narrative review of such knowledge is therefore undertaken here in order to add to research appraised in the extensive review and assist with devising research questions.

Contemporary research and opinion is reviewed on the following reproductive health indicators, bio-markers and stages; X and Y sperm chromosome proportion, sperm quality, DNA sperm fragmentation, testosterone levels, epigenetic environment, miscarriages (spontaneous abortion), and birth outcomes. This incorporates epidemiological inquiry similar to sex ratio research, with air pollution monitoring and serum measurement of dioxins and PCBs. Significantly though these studies also include biological measurements of sperm, such as sperm chromatin structure assay, clinical fertility investigations, and other potentially 'toxic' chemicals, Bisphenol A and polyfluronated hydrocarbons. A large component of this research is derived from 4 major environmental and reproductive health research projects, the Seveso Health Studies (including male and female cohorts), the E.U. INUENDO project (Biopersistent Organochlorines in Diet and Human Fertility: Epidemiologic Studies of Time to Pregnancy and Semen Quality in Inuit and European Populations), The Teplice Study in the Czech Republic, and the Swedish Families Fishermen's Cohort.

3.3.1 Sperm XY: XX Chromosome Ratio

The proportion of X and Y chromosome bearing spermatozoa amongst the male population is consistently accepted as not a significant departure from a 50:50 percentage (Boklage 2005). Researchers in Sweden however, have theorised that alterations in the Y:X chromosome ratio from oestrogenic and anti-androgenic substances, principally PCBs, could have a knock-on effect on birth sex ratios in certain populations with the 'preferential development' of one sex of embryos. The invention of fluorescence in situ hybridization (FISH) in sex-selection clinical practice has permitted analysis of Y:X chromosome ratio in epidemiological studies investigating such a hypothesis. Comparison of X:Y sperm proportions and exposure to POPs for East and West Coast fishermen in Sweden revealed an association between CB-153 and p,p'-DDE serum levels and greater proportions of ejaculated Y-bearing spermatozoa (Tiido, Rignell-Hydbom et al. 2005). Exposure to POPs from the Baltic Sea and high consumption of fish for East Coast fishermen and their families in Sweden has been widely documented (Rignell-Hydbom, Axmon et al. 2007). A further comparative study was also undertaken on cohorts from Greenland, Poland (Warsaw), Ukraine (Khariv) and Sweden which reported a negative correlation of PCB-153 and Y-bearing spermatozoa in Warsaw in contrast to positive associations in Greenland (non-significant) and Sweden (significant) (Tiido, Rignell-Hydbom et al. 2006). One further study on the Swedish Fishermen's Families Cohort examined potential association with Mercury levels and the Y:X ratio with no statistical relationship discovered (Rignell-Hydbom, Axmon et al. 2007).

The validity of the Swedish results is established through the recruitment of cohorts for sperm quality investigation *out with* fertility clinics or exclusively fertile couples. There is therefore far less potential for selection bias in Sweden where as the Poland and Ukraine samples were taken from partners of pregnant women. The difficulty for the Swedish cohort findings however, particularly in links to sex ratio alteration, is the insufficient explanation of biological mechanisms with exception of mention of Potashnik's DBCP study. The findings though do contribute to further understanding of specifically PCB and POP involvement in human reproductive changes. This also takes into account the previous reports on sex ratio alterations discussed in Chapter 3 review, which all lend support to a possible bi-phasic dose-response phenomenon. This presents as acute PCB and/or dioxin exposure equating with decline in male births, demonstrated in the Seveso research, and a rise in sex ratio when long-term chronic doses occur (Mocarelli, Gerthoux et al. 2000; Karmaus, Huang et al. 2002). Potentially, this could be mediated by changes in the Y:X sperm chromosome ratio amongst exposed male populations.

3.3.2 DNA sperm fragmentation

Sperm chromatin integrity is the extent of packaged chromatin structure in spermatozoa. The extent of DNA damage to this structure is known as the DNA fragmentation index (%DFI) and is tested by the sperm chromatin structure assay (SCSA) or alternatively through terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL). It is now understood that sperm chromatin integrity has an important influence on reproductive outcomes, more specifically on embryo implantation and morphology, and/or gene mutation. The DNA fragmentation index is increasingly favoured as a defacto marker of sperm quality, both for clinical fertility research and epidemiology, as it quantifies the cellular and functional properties of spermatozoa.

A crucial study using the DNA fragmentation index for investigating sperm quality and air pollution was undertaken in the highly polluted district of Teplice in the Czech Republic. Rubes, Selevan et al. (2005) establish a significant association for young men in the area of exposure during high periods to air pollution and DNA sperm damage. Although a relatively small sample of 36 young men is recruited, a comprehensive number of other factors are accounted for including smoking, alcohol, caffeine, fever and loose fit of underwear. Blood samples were also taken to measure levels of known endocrine disruptor chemicals lead, cadmium and mercury. These and the multiple collections of semen samples over low and high periods of pollution were methodological improvements on an earlier study in Teplice which reported similar findings (Selevan, Borkovec et al. 2000). Follow up longitudinal study has also suggested an environmentally mediated genetic link with a greater propensity for DNA fragmentation amongst exposed males with the homozygous null for glutathione-S-transferase M1 (GSTM1) genotype (Rubes, Selevan et al. 2007).

Investigations of DNA fragmentation have also been conducted as part of the INUENDO and Swedish Fishermen's Families projects with varying results. Associations were established with CB-153 serum levels and percentage of DFI for the Swedish East Coast fishermen and Ukrainian cohort, whereas Inuit men from Greenland and men from Warsaw did not display high levels of DNA sperm damage. There were no positive associations with DDE serum levels (Spano, Toft et al. 2005). The TUNEL assay was also measured in further INUENDO research replicating the earlier findings of geographical difference and the potential PCB linkage to sperm dysfunction across all the European countries (Stronati, Manicardi et al. 2006). Researchers have speculated that Inuit 'immunity' to sperm DNA damage for a highly POP exposed population group may be genetically related (Spano, Toft et al. 2005).

The strength of this environmental epidemiological research lies with the lack of selection bias which weakened previous sperm quality analyses from infertility clinics. This is particularly the case for the Swedish cohort where recruitment is the most representative and noticeably PCB associations are strongest. The work on sperm DNA damage also incorporates *both* potential endocrine disrupting processes and environmentally mediated genetic changes, with sulphur dioxide and particulate matter measured from the combustion of coal in Teplice.

An important recent study was also conducted on Bisphenol A (BPA), a prominent endocrine disrupting compound, with results showing a 10% rise in DNA sperm damage with every inter-quartile range increase of urinary BPA (Meeker, Ehrlich et al. 2010). Caution is exercised with such a finding as there are no confirmatory studies as yet and representativeness from one study is questionable given recruitment was only from men of sub fertile couples.

Clinical research and reviews on sperm chromatin integrity and reproductive outcomes is also relevant to the sex ratio subject. There is strong evidence from multiple studies that sperm DNA fragmentation has a deleterious effect on male fertility and embryonic development. The chances of fertilisation both naturally and through artificial insemination are significantly decreased with DNA sperm damage to the male partner according to andrology studies (Rybar, Markova et al. 2009). Most recently research has suggested that this manifests as a late paternal effect during or after embryo implantation (Frydman, Prisant et al. 2008). Coupled with this evidence is substantial research indicating poor chromatin integrity with recurrent pregnancy loss and occurrence of spontaneous abortion (Brahem, Mehdi et al. 2011). A further important finding from a review of paternal age and reproduction is a consistent association of rising paternal age and increased DNA sperm damage amongst sperm donors and in men of infertile couples (Sartorius and Nieschlag 2010).

Lifestyle and health factors have also been examined for sperm quality parameters with a limited number including DNA fragmentation. Pre-natal exposure to cigarette smoking is not associated with a decline in sperm chromatin integrity according to two studies whilst maternal weight is weakly correlated with higher DFI values. Hakonsen, Spano et al. (2012), in a comprehensive analysis of sons aged 18-21 identified from the Danish Civil Registration System exposed to varying degrees of maternal smoking, established higher DFI levels with maternal age, maternal alcohol and coffee consumption and parental time to pregnancy, though none of these associations were statistically significant .

3.3.3 Reproductive Hormones

As discussed in Chapter 2, James proposes that mammalian sex determination is linked to the balance of hormones for conceiving couples, namely testosterone and gonadotrophin levels (James 2008). Scientific interest in the hormone disrupting properties of particular chemicals includes evaluating whether reproductive hormone levels in humans alter as a result of certain exposures. Experimental animal studies have frequently demonstrated the estrogenic, anti-estrogenic and androgen interfering properties of persistent organophalogen pollutants such as dioxins, PCBs and DDEs. As part of the INUENDO project, a multiple analysis of serum levels in concentrations of POPs and hormones was performed with considerable geographical differences in the results (Giwercman, Rignell-Hydbom et al. 2006). The most significant associations were established in Kharkiv in the Ukraine, including both PCB and DDT concentrations and sex-hormone binding globulin (SHBG), and PCB with luteinizing hormone function. In Greenland a weak association was established with DDE levels and testosterone, whilst the highest exposed group to CB-153 also presented with high luteinizing hormone function. In contrast the Swedish participants did not exhibit any significant associations or irregularities by category according to POP exposure (Giwercman, Rignell-Hydbom et al. 2006).

One further study was also completed on 703 Native American men and women, all recruited by random selection of households rather than through fertility or ante-natal clinics (Goncharov, Rej et al. 2009). A notable finding from this research was a negative correlation with some PCB congeners and serum testosterone for males but in contrast no associations were established for women. Corroboration of such results is difficult to trace in further research, with a negative association between PCB and SHBG in a Swedish study and no associations established in the Seveso cohorts of testosterone levels and serum dioxin concentrations (Rignell-Hydbom, Rylander et al. 2004; Mocarelli, Gerthoux et al. 2008). Overall there is a very mixed picture with regard to findings on POP exposure and reproductive hormones potentially attributed to multiple testing, different exposures, potential selection biases and different genetic profiles.

3.3.4 Sperm Quality (exc. DNA Fragmentation)

Declining sperm quality in industrialised countries over the last 50 years, both in terms of counts and motility, has been reported from meta-analyses of region-specific cohort and cross-sectional studies (Carlsen, Giwercman et al. 1992; Swan, Elkin et al. 2000; Rolland, Le Moal et al. 2013). This temporal trend is attributed by some scientists to endocrine disruptor pollution, forming one part of *testicular dysgenesis syndrome* (TDS) which also includes increased testicular cancer rates and incidences of chryptorchidism and hypospadias. The proposed phenomenon has been disputed by some commentators though, as selection bias from such large meta-analyses inevitably compromise results (Safe 2000). A lot of recruitment of samples for the individual studies quantified in analyses is solely from sperm banks that establish quality criteria and age restrictions for sperm donation. Joffe (2008) for example suggests that geographical differences in sperm quality are more categorical than a 'global' declining trend with deterioration most likely confined to particular localities.

Immediate cohort recruitment at the Yucheng cooking oil contamination episode in 1979 has permitted inter-generational monitoring of reproductive effects from PCBs. The highly-cited Taiwanese study involved examining health outcomes of exposed men and women who were in-utero at the time of their mother's cooking oil consumption. For males, semen volume and counts did not differ to a control group, however sperm motility was reduced and abnormal morphology was significantly greater (Guo, Hsu et al. 2000). Detailed examination of semen samples also revealed reductions in penetration and bindings to hamster oocytes, and changes in sex hormones homeostasis (Guo, Hsu et al. 2000). Similar results for foetal exposure were reported from dioxin releases detailed in Seveso studies. An important finding was an age-dependent effect, with men exposed as adults not displaying adverse sperm parameters whilst for those exposed during puberty semen counts and motility increased (Mocarelli, Gerthoux et al. 2008).

Spatial environmental epidemiological inquiry has also emerged as new research on sperm quality. As per the Teplice research, U.S. studies conduct models of time-lagged local air quality concentrations with local sperm samples and test for statistical associations. Results are variable, with one study in North Carolina reporting no significant sperm quality deterioration from O^3 and PM exposures whilst research in Utah revealed drops in sperm motility for the only pollutant measured that being particulate matter (Hammoud, Carrell et al. 2010).

3.3.5 Epigenetic Environment

A crucial stage for human reproduction and 'normal' gestation development is embryogenesis, occurring between conception and the clinical recognition of pregnancy at approximately 8 weeks. Embryogenesis is the biological process of embryo formation eventually resulting in the development of the foetus. Starting with fertilization of the ovum (or egg) by sperm, the zygote (fertilised ovum) undergoes rapid mitotic divisions and cellular differentiation. An important opinion paper, published in Human Reproduction in 2005, explores embryogenesis in the context of changes to secondary sex ratio (Boklage 2005). Such a focus is rare in the subject literature with the scoping review revealing a scarcity of population health research connected to this reproductive biological stage. Boklage (2005) disputes the primary ‘conception’ sex ratio as determining the final live birth sex ratio citing the repeated evidence in many fertility studies of almost equal proportion of XX and XY chromosome bearing sperm. It is proposed that male embryos develop more efficiently and faster leaving an excess loss of female embryos in the transition from fertilisation to foetal development. This is attributed to genome imprinting and paternal epigenetic factors with corroborative evidence that includes genetics theory, experimental results of paternally imprinted X chromosome retarding early development of mice, and consistent sex ratio differences by paternal factors for inter-racial births (Boklage 2005).

The ‘male embryo epigenetic efficiency’ thesis is instructive for this investigation in two key areas. Firstly, a possible paternal link to secondary sex ratio through sperm DNA changes and genome imprinting at fertilisation; thus suggestive of a sentinel marker for male reproductive health. Secondly, environmentally mediated genetic changes in sperm may be applicable to Boklage’s model of alterations to sex ratio during embryogenesis.

3.3.6 Foetal Mortality: Miscarriage

According to clinical evidence about 15% of recognised pregnancies result in spontaneous abortions and by including very early foetal loss (unrecognised pregnancies) there are estimates of up to 50% of conceptions that do not lead to live births. Miscarriage, or the medical term of spontaneous abortion, is commonly defined as pregnancy loss occurring before 20 weeks of gestation. A multitude of factors and toxins have been linked to pregnancy loss pre-dominantly relating to maternal health or exposure. These include advancing age, infections, uncontrolled diabetes, tobacco, caffeine, alcohol, diet and obesity as well as other environmental toxins such as mercury, lead and benzene (Maconochie, Doyle et al. 2007). Recently, paternal links to miscarriages are increasingly being examined in obstetric and embryological research with sperm abnormalities and chromosomal defects theorised as responsible for foetal mortality. Male age is receiving considerable attention in contemporary research with one review stating that 'there is clear evidence for an increasing risk of miscarriage and fetal death with higher paternal age' (Sartorius and Nieschlag 2010). This may be attributed to morphological and genetic changes in sperm with advancing age such as point mutations, greater frequency of aneuploidies and DNA fragmentation (Slama, Bouyer et al. 2005; Sartorius and Nieschlag 2010). As an example, a rigorous case-control study of 92,408 births in Jerusalem found that women with partners aged 35 years or older were nearly 3 times more likely to have miscarriages compared with women conceiving with 25 year old or younger men (Kleinhaus, Perrin et al. 2006).

Evidence linking pollution with increased incidence of spontaneous abortions is reasonably scant, with the exception of the methyl mercury poisoning described in Chapter 2. In the cases of Taiwanese oil poisoning, Seveso, Great Lakes recreational fishing, and Swedish Fishermen's Family cohort no associations have been established (Toft, Hagmar et al. 2004). A study of reproductive outcomes amongst U.S. factory workers highly exposed to dioxins from Agent Orange manufacture also did not establish an increased risk in spontaneous abortion. A further study on Agent Orange exposure amongst Vietnam Veterans however revealed results of foetal loss for their partners if Veterans were only subject to background or low levels of contamination.

A predominance of the male sex of foetus is a key characteristic of pregnancy loss and also significant for live birth sex ratio trends. For an insight into this phenomenon it is helpful to firstly examine ecological studies on pre-term births. Zeitlin, Saurel-Cubizolles et al. (2002) extracted and analysed data from across 4 large health registries/surveys covering births in numerous global locations and concluded that male birth proportion declines with increasing gestation. Their results also show male excess is greatest for spontaneous preterm births, those occurring before 33 weeks. In a pan-European data-set which included normal term births, and live singleton and stillbirths between 22 and 36 weeks of gestation, the term proportion for male births was 52.1% compared to early pre-term births of 55.8%. Latest obstetrics and gynaecological research strongly suggests this male excess extends to spontaneous abortions and is in fact even more pronounced during the early stages of pregnancy. The greater vulnerability of the male foetus has been widely documented however empirical evidence of a 'male' skewed sex ratio for aborted embryos is not available. Hassold (1983) notes the impossibility of identifying the sex of aborted foetuses owing to inadequate genital development but conclude on a best estimate from previous research of a 115:100 primary sex ratio, the sex ratio at the time of implantation.

3.3.7 Birth Outcomes: Low Birth Weight and Small for Gestational Age

Literature and research focusing on factors affecting birth outcomes is extensive and almost unanimously concerns maternal health. Systematic reviews and meta-analyses and reviews have been undertaken which serve as useful summaries of research on smoking, alcohol and socio-economic status in connection to birth outcomes. Maternal tobacco smoking and adverse birth outcomes, including low birth weight and small for gestational age, are well established associations from long standing peri-natal health research. Further, meta-analysis of studies on environmental tobacco exposure, or passive maternal smoking, reveals 60g less birth weight to exposed babies but no changes to occurrence of small for gestational age (SGA) (Salmasi, Grady et al. 2010). The influence of alcohol on peri-natal outcomes is also researched widely and has received substantial recent media attention. Patra, Bakker et al. (2011) conclude, from a systematic review and meta-analysis of 36 studies, that a dose-response relationship exists for alcohol and low birth weight and SGA, whereby 1.5 drinks/day had no effect whilst greater consumption led to increasing risks of these adverse birth outcomes. There is presently substantial work being undertaken on maternal binge drinking and heavy alcohol consumption and intra-uterine effects, particularly cases of foetal alcohol syndrome.

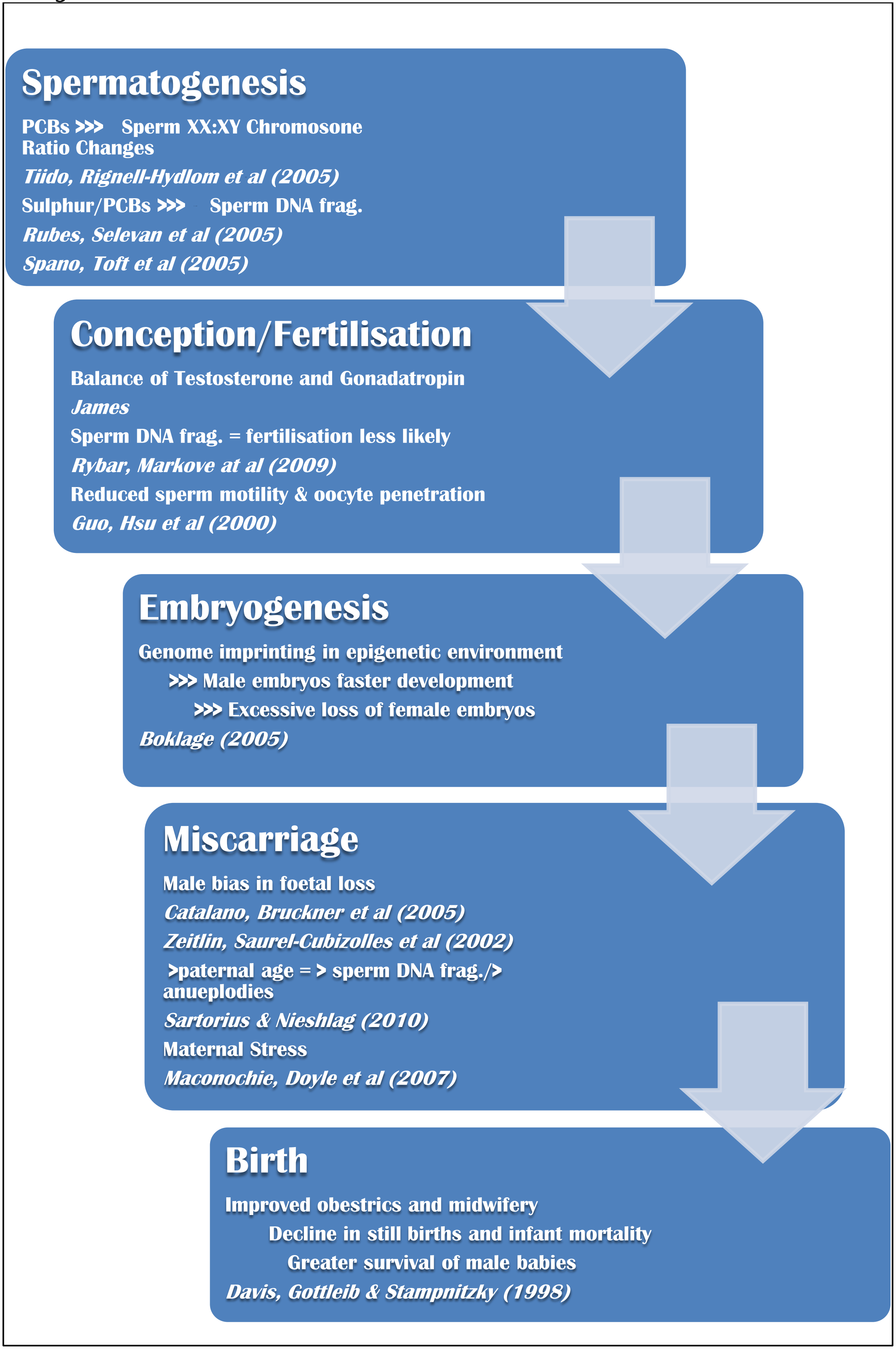
Socio-economic disparity and poor health status is a consistent theme for social epidemiology and this also persists for birth outcomes. In synthesis of evidence from 93 of 106 studies, measurement of socio-economic disadvantage was significantly associated with detrimental birth outcomes, including low birth weight and small for gestational age (Blumenshine, Egerter et al. 2010). Geographical analyses, using area-based neighbourhood data in the U.S. and Canada, have examined further factors associated with socio-economic disadvantage which may be responsible for maternal and peri-natal health discrepancies. Exposure to violent crime, perceived insecurity of neighbourhood and physical deterioration in a neighbourhood are factors which have all been positively associated with low birth weight and SGA (Farley, Mason et al. 2006; Messer, Kaufman et al. 2006; Auger, Daniel et al. 2008). Further, quality of residential built environment based on 7 indices in census data is associated with improved birth outcomes in North Carolina (Miranda, Messer et al. 2012). Although quantifying such social phenomena at large metropolitan scales has repercussions for validity, results could suggest exposure to chronic stress for mothers living in deprived areas with a resultant increase in risk of preterm birth (Elo, Culhane et al. 2009). Daily exposure to threatening and poor physical environments in neighbourhoods which initiates stress responses and adverse health outcomes has been developed theoretically and empirically in epidemiological study with links made to cardiac and respiratory disease, and reduced resistance to cancers and infections (Hill, Ross et al. 2005).

Consistent with other indicators, epidemiological study on the effects of pollution and birth measurement is unequivocal although a sizeable majority of studies show statistical relationships with exposure measures and birth outcomes. Caution must be exercised for some results as these only include surrogate measures of exposure, such as food frequency questionnaires in Denmark and geographical comparisons of the East and West coasts in Sweden (Rylander, Stromberg et al. 1995; Halldorsson, Meltzer et al. 2007). A more detailed study conducted on 494 mothers and newborns in Valencia measured DDE concentrations in umbilical cord serum and birth outcomes, including birth weight, length and head circumference (Lopez-Espinosa, Murcia et al. 2011). Co-variates were accounted for in the study and results revealed decreases in birth weight, length and head circumference for tenfold increase in 4,4'DDT concentration. Further, a retrospective cohort study of women residing in contaminated areas of the Seveso event showed birth weight and SGA alterations for pregnancies within 8 years of exposure (Eskenazi, Mocarelli et al. 2003). Contradictory results emerge for low level exposure, most frequently analysis on PCBs, although a recent study found associations with increased SGA and some PCB congeners from stored serum samples of U.S. mothers from 1960 to 1963 (Kezios, Liu et al. 2012).

3.3.8 Generative Mechanisms of Secondary Sex Ratio Alteration

The key generative mechanisms for sex ratio adjustment derived from a small narrative review of available literature and research is presented thematically in Figure 2.

Figure 2: Generative Mechanisms



The diagram is an attempt to map potential stages of alteration in the final live birth sex ratio by displaying the biological chronological process of reproduction - spermatogenesis, conception/fertilisation, embryogenesis, pregnancy and birth. Synthesis of such knowledge can assist with informing preliminary research questions posed in Chapter 2 and also present further questions of inquiry in this study.

As has already been documented there is extensive experimental and public health evidence for a deterioration in spermatogenesis amongst Western males in modern times. One objective of this and the literature review is to isolate which crucial sperm quality parameters likely effect subsequent determination of sex of offspring. Reporting on proportions of XX/XY bearing spermatozoa through FISH analysis is elucidatory when assimilating such findings with sex ratio research on PCB exposure. The Swedish results of Y bearing excess in the East Coast fishermen population is potentially instructive for the increased sex ratio skewing amongst Great Lakes Michigan fish-eaters discussed previously. It is interesting to note, however, that the heavily PCB exposed indigenous Arctic population cohorts do not display significantly altered X:Y bearing proportions in sperm. The potential bi-phasic dose-response hypothesis from PCB exposure is thus partially sustained though it would be conjectural to extrapolate to wider male birth proportion trends. Evidence would suggest that acute and large chronic exposure is localised in terms of male reproductive health impacts. Clinical research has consistently shown an almost equal balance of XX/XY bearing spermatozoa in representative male population groups.

Recent findings on poor sperm chromatin structural integrity (DNA fragmentation) associated with PCB exposure and local sulphur air pollution is significant. Population based studies are replicating observations of PCB association with a number of male reproductive parameters (X:Y bearing sperm proportions, DNA fragmentation, sex ratio) vis-a-vis other chemical agents e.g. DDT metabolites and mercury. The INUENDO study results of elevated indices for the Swedish East Coast and Ukraine of sperm measurement methods, DNA fragmentation and TUNEL assays, further demonstrates likelihood of a PCB linkage to genetic sperm dysfunction. Further, it is feasible that the consistent association of increased parental age and DNA structural sperm damage may partly arise from cumulative PCB lifetime exposure.

Adoption of the DNA fragmentation index in epidemiological study has been integral to development on an alternative thesis to endocrine disruption, that of environmentally mediated genetic alteration to sperm. Sulphur is not identified as an endocrine disruptor in current databases which opens the possibility of a plethora of common air pollutants linked to detrimental reproductive health impacts e.g. particulate matter, nitrous oxide. The hypothesis is chiefly expounded by Michael Joffe, who contests that endocrine disruption and TDS is not reflected in the associated descriptive epidemiology. Reiterating the methodological inconsistencies of studies included in Carlsen's sperm quality meta-analysis and emphasising the *earlier* presentation of testicular cancer increases before production of hormone disrupting compounds such as phthalates in 1930s, Joffe presents assertive arguments for a genetic component to TDS. This hypothesis in simplified form, which incorporates pathogenesis and meiosis of cells, is one of an initial environmental insult to the foetus and damage to structure of the DNA germ line with ever increasing duplications and deletions over time and across generations (Joffe 2011). Genetic damage in male reproduction therefore has an explanation for 'anomalies' of unremarkable sperm parameters in polluted regions, such as Greenland, and presents an inter-generational temporal framework for understanding potential sentinel changes in reproductive health.

As documented earlier, structurally DNA damaged sperm can compromise fertilisation and further, early pregnancy loss in Western populations is likely to have risen in recent times given the increasing trend of fertility problems amongst couples. A parallel decline in male proportion of births may therefore be symptomatic of such mechanistic alterations at the embryo implantation stage of reproduction. Whilst these propositions are very speculative the question of population and environmental determinants of environmentally mediated genetic changes in sperm is also pertinent to secondary sex ratio adjustment.

Adult men, exposed peri-nally during the Yucheng 'rice oil' incident exhibit decreased sperm motility and incapacity to fertilise oocytes from endocrine disruption. These findings, however, have not been replicated and may solely reflect very unusual high dose-response events. Review of epidemiological studies on reproductive hormones gives little vindication of James's 'balance of testosterone/gonadatrophin' hypothesis though realistically such a theory, given logistical and ethical difficulties of measuring hormone levels at conception, is very difficult to sustain or refute with evidence. The importance of this hypothesis is the emphasis on a combined, parental mechanism for primary sex ratio determination, thus illustrating the complexity of factors implicated which can vary according to time and parental health status. Frequently other commentary focuses on either a male or female catalysing mechanism which may be too simplistic an explanation for the derivation sex of an embryo and birth.

Male mediation of secondary sex ratio in the epigenetic environment via genome imprinting is also an important thesis for testing and exploration in research. A decline in the male proportion of births in some industrialised countries may be a marker of epigenetic processes in populations, potentially linked to the development of early life disease paradigm and plasticity modulations in embryos (Gluckman, Hanson et al. 2007) and the environmentally mediated genetic changes in sperm.

In terms of first trimester pregnancy and miscarriage, the narrative review details substantive evidence of male bias in foetal loss. Accepting this, the question then arises as to whether miscarriage has increased over the last 40 to 50 years thus resulting in a drop in the male proportion of births. Acquiring data to address such a proposition is problematic however, given the unreliability of early pregnancy loss measurement. Notwithstanding this, well-designed, tightly focused cohort studies have identified advanced parental age and maternal stress as associated with increases in spontaneous abortion. Meanwhile there is limited evidence of an early pregnancy loss and environmental hazard linkage according to studies conducted over the same cohorts examined for other reproductive outcomes e.g. Seveso, Yucheng.

Small for gestational age births and low weight births are indicators which has been extensively analysed with socio-economic, health-related and environmental factors. Their connectivity as a mechanism for secondary sex ratio alteration, based on the greater vulnerability of male babies to growth retardation and defects, is the least definitive of all the reproductive stages. However, contemporary epidemiological theory and research on chronic and place-based stress is identified in the review which may be commensurate with the stress and sex ratio hypothesis. Further, a key focus in peri-natal epidemiology of smoking in pregnancy and birth outcome is also pertinent to sex ratio inquiry.

3.4 Scoping Review: Synthesis and Research Implication

This, and the preceding chapter, summarise and appraise the evidence of factors influencing human sex ratio. The scoping review illustrates the array of scientific perspectives which have been adopted in attempting to understand the declining male birth proportion phenomenon. This permits a series of research questions to be expressed which underpin the investigation for the rest of the thesis. Subsequently, in Chapter 4, discrete, testable hypotheses are formulated which draw from these reviews and fall within the confines of data, resources and time available for a post-graduate 3 year thesis.

Synthesis of such multi-disciplinary research on sex ratio change is challenging given the variability in method types attached to disciplines. Although potential determinants are categorised as either environmental or socio-economic and demographic or health-related, there is a distinct possibility that any combination of the factors concerned may explain male birth proportion decline in industrialised countries. This premise is pivotal to the objective of this research, to undertake a multi-variate and spatial analysis of potential determinants of birth sex ratio in Scotland. Numerous mitigating factors affecting male birth proportion decline may also be significant, which is a rationale for studies identifying upward skewing in sex ratio being included in this review.

The literature review on endocrine disrupting chemicals reports inconsistent results in sex ratio alteration although, as discussed, exposure assessment varies across the studies. It is therefore difficult to extrapolate from these cohort investigations on specific substances to a link to the wider population trends of lower male birth proportion. Importantly though, only a limited number of endocrine disruptors are measured in this discrete area of research with 870 potential chemical agents with hormone disrupting properties currently identified in peer-reviewed scientific studies (The Endocrine Disruptor Exchange 2012). For example, Bisphenol A is not identified in the review but, as it is ubiquitous in plastic consumer products, it is currently extensively investigated in other research and engendering much scientific debate. The key results related to environmental toxicology in Chapter 2 include the following; (i) acute high level dose effects from dioxin as per Seveso study; (ii) possible downward and upward alterations in sex ratio from PCBs potentially varying according to chronic release or a particular congener; (iii) a potential male route of exposure evidenced through the Seveso investigation identifying young men as vulnerable and the Russian pesticide workers study, and; (iv) a robustly designed study of long term chronic PCB exposure demonstrating a possible female 'route' effect. The potential bi-phasic effect of PCBs described in (ii) above supports the changes in balance of XX and XY bearing spermatozoa for Swedish East Coast fishermen.

It should also be noted, however, that well-designed toxicological investigations, such as on PCBs in breast milk and lead pollution, found no effect is measured on sex ratio. These sex ratio changes or unremarkable results may reflect different endocrine disrupting properties for particular compounds or congeners, or such effects may be dependent on dose or timing of exposure. Further, the new paradigm in toxicology emerging from the environmental endocrine hypothesis, of a combination of low dose, mixture/interaction of chemicals, and timing of teratogenic effects, potentially on human health and demonstrated in animals, is only modelled in a very minimal way in the studies reviewed. Superior designs of occupational studies are opportune in this regard given the potential capacity to measure multiple exposures, either by biomarkers and/or new survey methods e.g. OccIDEAS (Behrens, Mester et al. 2012). Further, a relatively new paradigm of environmentally mediated genetic change in sperm should also be considered with regard to male birth proportion decline and male reproductive health, particularly as its mediation is potentially inter-generational and may relate to the vulnerability of young men to such pollution impacts. Environmental insult to women, with particular sensitivity during the time of pregnancy is also of vital interest for studies investigating reproductive health parameters, including secondary sex ratio.

The geographical studies of pesticide exposure, air pollution, radioactivity and local proximity reveal no definitive consistent evidence for linking environmental pollution to national male birth proportion declines. However, when considering both the individual toxicology and geographical cases, such as dioxins in Seveso and PCBs in the Russian Arctic, and local multiple media pollution e.g. Sarnia, there are prominent examples of localised sex ratio alteration. Further, where acute contamination is very high, as is illustrated with radioactivity and Chernobyl, there is reasonable evidence for local sentinel reproductive health events in the form of sex ratio skewing. The question remains however, about the extent to which this transposes to sizeable populations experiencing a trend of male birth proportion over a far longer period. In cases of local sentinel events, knowledge can be improved through follow-up studies, such as well-designed epidemiological research which can incorporate recruitment of cohorts and also community-based reproductive health impact assessments currently being conducted in Sarnia, Ontario. Local investigations which integrate different disciplines, such as sociology, toxicology and community health, can be adopted for such future research on sex ratio alteration (Van Larebeke et al. 2008). Notwithstanding this, there are also the methodological difficulties in modelling or measuring environmental exposure given the geographic and temporal variation and composition of multiple and potentially interacting pollutants.

More detailed exposure assessment is also imperative for future study as many of the ecological studies do not analyse relevant socio-economic statistics or longitudinal data which may be pivotal to understanding routes or timing of exposure. There is also a tendency to focus on one pollutant media (air) and route of exposure (respiratory) in the geographical, ecological and time-series analyses. Other routes of exposure, such as ingestion and dermal transference, may confound some of the findings on sex ratio skewing that have received scholarly and media attention. Further, chance cannot be eliminated from some remarkable results given the small numbers or sample sizes involved and the stochastic nature of sex ratio trends. A possible avenue to overcome such issues is to undertake assessment across diverse geographical units in addressing the modifiable areal unit problem (discussed in next chapter). The increasing use of Geographical Information Systems in future research can address such problems given its massive data storage capacity and processing speed, potentially improving exposure assessment for reproductive health and sex ratio studies overall. Despite the weakness of lack of statistical power from small area investigations however, release of births data and reporting of it affords the opportunity of ‘honing in’ on long-standing areas of pollution concern for researchers and environmental monitoring teams. The sentinel attribute to sex ratio monitoring of whole birthing populations in small areas, in contrast to very isolated incidences of other reproductive indicators e.g. low weight births, is important in this regard.

Evidence on socio-economic factors chiefly concerns the Trivers-Willard hypothesis, and some studies reviewed are subject to flawed method whilst other research produces results which are contradictory to the adaptive biological claim of sex ratio modulation. Given also that very large population studies have been repeatedly analysed with the relevant variables, an assumption could be made that male birth proportion change is *not* linked to social class or socio-economic status. Exceptions to this may be where occupational groups are exposed to hazardous chemicals, and/or health behaviour, such as smoking and poor nutrition in lower income cohorts, which may be linked to lower male births. These factors can correlate with socio-economic status but biological deterministic research on adaptive 'social class' behaviour alluding to natural selection appears somewhat questionable and out-dated. It is certainly questionable, despite an extensive number of studies, as to whether socio-economic status is a predictor of sex ratio. As such, inclusion of socio-economic measurements as confounders in environmental studies may not be warranted. Socio-economic status, however, may act as a proxy for some exposures, such as tobacco and common non-endocrine disrupting pollution, such as Nitrogen Dioxide, with concentrations larger in poorer areas (Briggs, Abellan et al. 2008).

There are, however, substantial findings from large demographic statistical studies to suggest parental age has an influence on sex ratio, with ageing leading to a decline in male birth proportion. A generative mechanism - illustrated for secondary sex ratio of advancing parental age and reproductive effects, particularly increasing sperm DNA fragmentation for fathers and greater miscarriages amongst couples is also pertinent in this regard. There is substantial evidence therefore from both a sex ratio inquiry, and peri-natal and andrological research, that parental age may be a key determinant of male: female birth proportion in populations.

This review also highlights a sociological perspective on sex ratio change, which is rarely discussed in research articles and commentaries. As emphasised in the Israeli study on religious law, socio-cultural norms may influence timing of conception and thus sex ratio. Significantly, frequency of intercourse and conception time is also considered in the context of population stress and parental age, and sociological factors could be influencing changes in sexual behaviour in industrialised countries. The recurrence of the timing of conception hypothesis in the review, linked to demographic, socio-cultural and health-related factors, would suggest further integrated study is required, such as on stress and frequency of coitus. Some studies excluded from the review however, detailing fertilisation techniques, are instructive in this regard as these results dictate that the timing of conception hypothesis is contradicted (Grant 1998). Despite this, research on socio-cultural and sociological factors influencing sexual behaviour and health is required in the context of secondary sex ratio decline, although it is likely fraught with ethical difficulties and systemic error, given the confidential nature of sexual relationships.

Health-related determinants such as heavy parental smoking and acute population stress provide a strong case for sex ratio decline in specific populations. Evidence also suggests tentatively, that calorific deficiency may be linked too, though all of these variables are subject to problems with auto-correlation. As mentioned previously, acute hazardous events which lead to population stress may also release pollutants which may be responsible for sex ratio alteration. The stress hypothesis studies and the almost unanimous accord with declining male births suggests future research could also be confirmed with contemporary work on stress, and perinatal and reproductive health, particularly relating to urban neighbourhoods of crime and deprivation, as well as pollution (Farley et al. 2006; Hill, Ross, & Angel 2005). The 'related indicators' findings of stress associated with greater occurrence of early pregnancy loss and miscarriage, combined with a confirmed trend for 'preferential' loss of male foetuses, is also revealing. With regard to smoking, the reliable Avon Longitudinal cohort would tend to negate previous findings of heavy tobacco use and male birth proportion decline but the recent Liverpool study, also accounting for confounders, re-emphasises the need for further research.

Assessment on any of these determinants would also benefit from multi-disciplinary reproductive health impact assessments which would include multi-variate modelling in order to 'draw-out' causes of local sex ratio decline or other deleterious effects. Further, a combined effect on population health from smoking and stress, and also environmental hazards and pollution, introduces the concept of vulnerable communities experiencing detrimental reproductive health changes. Valid measurements of socio-economic status can therefore delineate vulnerable populations to sex ratio alteration and reproductive health impacts, introducing the concept of 'environmental justice' to research on male birth proportion change. Notwithstanding, the Sarnia and other cases however, it should be emphasised that endocrine disruptor exposure is potentially most acute amongst higher socio-economic groups. The proliferation of hormone disrupting compounds in the manufacture of household products and personal cosmetics and higher income groups' greater capacity to consume such items is important in this regard. Caution should therefore be exercised in pursuing 'environmental justice' type research and including assumptions that endocrine disruptor pollution disproportionately affects the most disadvantaged populations.

Finally, advancing reproductive technology and peri-natal medical intervention are potential factors which represent gaps in research on male birth proportion in Western populations. The proposition that a rise in secondary sex ratio in the U.S. from the late 19th century to World War 2 can be attributed to the greater survivability of male babies from improved midwifery and obstetrics, together with the widespread adoption of reproductive sex selective technology and skewed sex ratios in Asian countries, suggest that conceptually at least peri-natal intervention can be linked to the comparative downward alteration in sex ratio. Research is scarce for example on any effects of induced abortion trends in various countries, with potential adjustments in sex ratio feasible from pregnancy terminations associated with medical detection of chromosomal abnormalities in the fetus, proportionately higher in males for Down's Syndrome. Such investigations may be ethically prohibitive and controversial, and the statistical effect in term of numbers involved is potentially minimal. The influence of advancing reproductive technology though and its adoption or non-adoption based on sociological structure or agency, coupled with other socio-economic and environmental factors already described, should not be ignored in debate on the declining male birth proportion phenomenon.

3.5 Research Questions

Synthesis of research on male birth proportion in this chapter identifies not only gaps in knowledge but also implications for research which could potentially be conducted in this doctoral study. The following research questions emerge from this review:

- *Is endocrine disruptor pollution responsible for the decline in male birth proportion in industrialised countries and/or sex ratio skewing in particular localities?*

- *Is the Trivers-Willard hypothesis and socio-economic status a useful and reliable predictor of sex ratio?*

- *Is there continued evidence of health behaviour influencing male birth proportion, particularly in relation to smoking and stress?*

- *Has sex ratio inquiry in Western countries focused too heavily on environmental and socio-economic factors rather than on biological mechanisms of human reproduction, such as medicalised interventions in reproduction?*

- *Is advanced parental age a predictor for predominance of female births and is the increasing social trend of older mothers and fathers in some European and North American countries responsible for sex ratio decline*

- *Are there dual or multiple effects on sex ratio from combinations of these environmental, sociological and behavioural factors which affect male birth proportion locally and/or nationally*

The results of the review and these research questions reflect the multi-factorial nature of the male birth proportion phenomenon. The directions of inquiry are wide-ranging and very difficult to tackle for one discrete research project. The following chapters address these questions through the selection of suitable research methods and mining of relevant and available data bases.

Chapter 4: Methods

4.1 Introduction

This chapter will outline the methods for this study, examine the quantitative and geo-statistical methods used and review the quality of the data analysed. The advantages and limitations of the methods adopted will be identified. Firstly, in order to generate null hypotheses to be tested a summary of the data-sets available in Scotland for analysis is given as well as an iteration of the overall scope and research agenda for the study. The methodological approach for the study is briefly stated and comment is made on the null hypotheses included and excluded for testing. The three stage analysis to be conducted is then described which incorporates; (i) descriptive statistics of birth sex ratio temporal trends and regional distribution in Scotland (ii) stratified analysis of birth sex ratios for small areas in Scotland and potential socio-economic and health–related determinants (iii) spatial analysis on small areas within the Central Scotland 'region' and associated birth sex ratios with endocrine disruptor pollution and socio-economic and health-related variables. Some of the methods adopted in this spatio-temporal analysis are then discussed (exposure assessment, geo-analytical inquiry) as well as limitations for method and data which need emphasising from the outset. An assessment is then given of the quality of the secondary data-sets available for analysis in Scotland which are sourced from government agencies and web-portals. Finally, a summary of ethical considerations is given.

4.2 Research Agenda and Data-Sets

Results of the systemically formed review illustrate the multi-factorial nature of the declining male birth proportion phenomenon. Attempting to deconstruct the constituent elements and multiple factors for secondary sex ratio is a pivotal and challenging objective for this study. Regression and geo-statistical analysis are common methods for investigating and attempting to 'unpack' multi-factorial 'problems'. This involves identifying important and significant statistical relationships amongst large groups of numerical and geographical data, in order to make explanations and/ or predictions of causes and effects in the 'real' world. Such studies in social science and demography rely on the use of large secondary data-sets covering national and regional populations, as shown by sex ratio research on parental age in the last chapter. These *ecological* studies are also common to epidemiological inquiry, and increasingly the addition of environmental data and geo-statistical analyses is advancing such research.

4.2.1 Data-Sets in Scotland

A further ecological study on sex ratio, which incorporates geo-analysis, is feasible in Scotland. This is due to the availability of relatively comprehensive and detailed public health and socio-economic data-sets, as well as environmental monitoring data. The abundance of societal data in Scotland and the UK stems from a long-standing governmental focus on strategic planning and housing initiatives in cities and more recently public policy addressing a very poor chronic health record, particularly in Scotland (Atkinson & Moon 1994). These data-sets include the Scottish Index of

Multiple Deprivation (SIMD), an equivalent indicator for socio-economic status in this study, and Scottish Public Health Observatory (SPHO) community profiles, which measure over 60 health indicators in neighbourhoods, districts and local authorities across Scotland. These data are replicated in the Scottish Neighbourhood Statistics (SNS) web-portal interface where multiple socio-economic, demographic, health-related and environmental measurements for small areas can be accessed.

In addition, Scotland shares in common with other European and North American countries, national environmental monitoring and pollution data which are publicly available. The Scottish Environmental Protection Agency (SEPA), the regulatory body for protecting environmental and human health in Scotland, is responsible for this data gathering. As part of its statutory remit SEPA monitors pollution across all media types, including air, surface water, waste water and land, as well as endeavouring to carry out enforcement duties. Assigned responsibilities for protecting the environment by the Scottish Government, it also functions under EU directives, including reporting to the European Pollutant Release and Transfer Register. (E-PRTR). SEPA performs this duty through the Scottish Pollutant Release Inventory (SPRI) which is an interactive public database of all pollution emissions beyond particular thresholds in Scotland. 215 pollutants are reported in the inventory and include a variety of chemicals identified as endocrine disruptors through scientific testing.

Accurate birth registration also exists in Scotland with about 60,000 to 80,000 birth events recorded per year and reported almost unanimously by home address of the mother. This birth data set is published by the General Register Office in Scotland

(GRO) and collected by local registrars in 228 registration districts. Scotland began one of the earliest forms of state birth registration in the world, commencing in 1865. These detailed registry data have recently been transformed to birth statistics on a government web-portal which are available for small and regional areas and includes total male and female births. Further, data are accessed by request from GRO (Scotland) officers and the Registrar General's Annual Reports, commencing from 1940, are also available at the central office. Additionally in Scotland the NHS Information Services Division (ISD) produces some of the most detailed and thorough birth morbidity and maternal health statistics in Europe.

Scotland's extensive collection of relevant data to secondary sex ratio inquiry, including comprehensive historical and geographical tabulations of sex at birth, offers opportunity for robust analyses and testing of hypotheses. An ecological study can be designed which compares exposures and socio-economic and demographic determinants in population groups with occurrence of health events, such as male birth proportion, in such groups. Further, an ambitious spatio-temporal approach, which also includes geo-statistical techniques and display of temporal trends, can potentially cast light on the secondary sex ratio phenomenon.

4.3 Scientific Method and Research Hypotheses

Research questions proposed following the review concentrate on five key possible influences on secondary sex ratio; endocrine disruptor pollution, advanced parental age, population stress, smoking and changing reproductive technologies. Valid and reliable population data in Scotland, described above, that includes a plethora of area-based indicators, can be conceptually equated with nearly all of these questions to generate hypotheses. The deductive method of scientific inquiry of interrogating and attempting to falsify hypotheses is thus the primary methodological approach for this study (Dunn 2012). Although in following standard scientific method there is also the qualification that a complex inter-play of factors is at work in the constitution of the human sex ratio – social as well as physical – which may not always be measured or unravelled in any study. Further, it is not possible in a study of this size and length and with limited resources to examine all the factors that may or may not contribute to birth ratios. The study is therefore focused on a number of key but manageable areas and the following null hypotheses which are tested for statistical association and/or spatial correlation.

4.3.1 Null Hypotheses

1. Pollutants identified as endocrine disruptors are not prevalent in regional areas or localities in Scotland with skewed low birth sex ratios, although levels and effects of those levels may be difficult to establish.
2. Birth sex ratio in small areas in Scotland is not affected by socio-economic status of the resident population in such areas.
3. The proportion of mothers over the age of 35 does not affect the secondary sex ratio in Scotland.
4. High maternal smoking prevalence does not affect the secondary sex ratio in Scotland.

5. Birth sex ratio in small areas in Scotland is not affected by chronic neighbourhood stress, in the form of high crime rates, alcohol hospitalisation and uptake of anti-anxiety and anti-depressant medication.
6. Economic hardship, poor health, neighbourhood stress and exposure to endocrine disruptor pollution do not cause a multi-factorial downward alteration in secondary sex ratio.

These hypotheses do not include all of the research questions outlined in the previous chapter as, firstly, there are not an inexhaustible number of indicators in the national data-sets and secondly, methodological consideration needs to be given to simplifying statistical models and spatial analysis in achieving *parsimony*. For scrutiny of the research question on modern medicalisation of reproduction, a cohort study or randomised control trial would be a more appropriate choice of type of study, similar to those described in the review. Demonstrating the null hypotheses above, however, could produce findings pertinent to the 'reproductive-specific' explanations of sex ratio change linked to evolving medical technologies.

4.4 Methods Summary (including Hypothesis Testing) and Data Processing

The analysis was conducted over 3 stages; (i) a review of temporal and regional trends in secondary sex ratio in Scotland (ii) stratified analysis and bi-variate correlation testing of small area-based data of birth sex ratio with, multiple deprivation, maternal age, maternal smoking and a proxy composite measure of neighbourhood stress. (iii)

GIS spatial analysis of small area birth sex ratios with modelled endocrine disruptor exposure for such areas, and also combined with the stress, deprivation and age indicators.

For stage (i), data were sourced at the General Register Office (Scotland), from the website (http://www.gro-scotland.gov.uk/statistics/), through GRO (Scotland) statisticians by request, and by a visit to the central records office in Corstorphine, Edinburgh. Some data were also extracted from the Scottish Neighbourhood Statistics birth tables which are compiled from all birth registrations geographically coded by GRO (Scotland). As per universal reporting of the secondary sex ratio, the formula applied for calculations was the following:

Birth Sex Ratio = Total Male Births (Region) / Total Births (Region)

The terms of either 'birth sex ratio' or 'secondary sex ratio' were applied in the graphs and tables and both equate with the frequently used North American 'male birth proportion' description. Figures computed included annual national, urban/rural geography, regional and local authority sex ratios, and cumulative regional, local authority area and parliamentary constituency ratios. Temporal trends were displayed for a variety of periods, from historical to most recent times as well as regional and locality variability, including areas of low skewed sex ratios.

4.4.1 Hypothesis Test of Two Proportions

Establishing significance of secondary sex ratio for particular years and areas was pursued through the hypothesis or Z Test of two proportions (large sample significance testing). These calculations were all performed using PASW statistics and the Macro syntax file for Large Samples significance test on two proportions (Pryce, 2005. Appendix A). The advantage of using this test was significance/non-significance is established based on sample sizes i.e. total birth numbers, rather than based just a crude ratio parameter, such as for binomial and chi-square tests. The Z values calculated from this test also permit comparison for non-significant samples. The two proportions tested included the birth sex ratio for a specific region or year from the graphs and tables with larger *independent* 'samples which included sex ratio for the Yorkshire & Humber English region and an earlier time period in Scotland (1974-1979). The Yorkshire and Humber region was selected as a 'control' population for comparison owing to its similar population structure and geography to Scotland. The English region contains approximately 4 million persons with similar number of births per year (about 50,000) as well as a concentration of metropolitan, industrial conurbations (Leeds – Bradford, Sheffield - Rotherham) and large rural hinterland. The cumulative 1974 to 1979 Scotland male birth and total birth figures were included as the 2nd larger proportion for testing significance of later years for the regions e.g. 1984, 1994. The two large samples are thus also independent of each other i.e. 1st regional sample is not a sub-set of 2nd sample. The null hypothesis stated was that the birth sex ratio in the 'subject' region or area was not different to the larger independent comparator sample for either Yorkshire and Humber or Scotland.

Stage 2 of the analysis involved use of health-related and socio-economic data from the 'Advanced Reporter' interactive data-base at Scottish Neighbourhood Statistics (SNS) to test 4 of the 6 hypotheses; population stress, maternal age, maternal smoking and multiple deprivation. The smallest area abstracted in SNS is the 'datazone' level (described 4.6.1) which was selected as the geographical unit of reference for the rest of the spatio-temporal analysis i.e. stages (ii) and (iii). These comprise 6,505 areas in Scotland formed by amalgamating census enumeration districts to then establish 'distinctive' neighbourhood zones with populations of between 600-1000 persons.

For the stratified (and spatial) analysis the following indicators from SNS were used for constructing the small-area based categorical variables including a composite index for neighbourhood stress:

- Deprivation : SIMD Vigintile (2006)
- Neighbourhood Stress : Percentage Prescribed Drugs for Depression, Anxiety or Psychosis (2004-07); No. of SIMD Crimes per 1000 persons (2007/08); Hospital Admissions for Drug Misuse, rate per 100,000 population (2001-04).

  *Neighbourhood Stress = (% Prescribed Drugs for Depression and Anxiety /10) * (SIMD Crime Rate + Hospital Admissions for Drug Misuse)*

- Maternal Age : Percentage of first time Mothers aged over 35 (2002-08)

The categorical variables were then cross-tabulated and aggregated in SPSS with Total Male Births and Total Births for datazones, permitting stratified calculation of secondary sex ratios for levels of multiple deprivation (20 vigintiles), neighbourhood stress, maternal smoking and ranges for percentage of maternal ages over 35. The two proportions test of significance was then performed on these sex ratios with the Yorkshire & Humber equivalent cumulative male birth proportion (1999-2008) used as the comparator sample. Pearson co-efficient and Spearman's Rank bi-variate correlation testing was also conducted on the dependent variable of 'datazone' sex ratio and each of the 4 socio-economic and health-related independent variables.

For the third stage, the spatial analysis is confined to a central region of Scotland of 6 local authority areas (Falkirk, South Lanarkshire, North Lanarkshire, Stirling, Fife, Clackmannanshire) which cover 1640 datazones. This region contains major sites of industrial pollution in Scotland, such as the Grangemouth INEOS complex and Longannet power facility, as well as large tracts of contaminated and remediated land, a legacy from Scotland's heavy manufacturing industry. There is also diversity of socio-economic characteristics in population with both urban and rural localities as well as deprived and non-deprived areas. Further justification for this approach and selection of this geographical area is given in the next section below. An index of *environmental endocrine exposure* was formulated involving the projection over datazone surfaces of total endocrine disruptor air pollution emissions in kilograms, for 2002 and 2004 to 2010 at SPRI registered emitter sites. The datazones were then stratified according to these levels of endocrine disruptor pollution to create the index. A simplified modelling of endocrine disruptor air pollution emissions was achieved by the following process:

1. Identification of Endocrine Disruptors on the SPRI Pollutant Search list. Cross-referencing of The Endocrine Disruption Exchange (TEDX) – *List of Potential Endocrine Disruptors* with SPRI Pollutant List (see Appendix B).
2. Systematic search of SPRI for Air Pollutant releases of identified Endocrine Disruptors for each year 2002 to 2010 (no records 2003) by 6 local authorities. Recording of cumulative total emissions (kg) for each site listed.

3. Grid reference obtained for each SPRI emission site using address and postcode from search results and www.gridreferencefinder.com. X and Y co-ordinates plotted on ArcMap.
4. Simplified modelling of source air pollution by approximating from (i) Met Office wind-roses and (ii) Air Quality scenarios from Environmental Statements on Grangemouth Renewable Plant (2012) and Dunbar Energy from Waste Facility (2008). 3 polygons created using ArcGIS of wind-rose directions (degrees) and predicted distance of pollutants (metres) for SPRI emission sites in (i) Falirk and Clackmannanshire (ii) Stirling, North Lanarkshire and South Lanarkshire (iii) Fife
5. Projection of air pollution polygons layer onto Central Region datazone layer to identify 'air polluted' datazones.

Overlay maps in ArcGIS are then produced of the environmental exposure index and sex ratio significance for the 1640 datazones, and combinations of the exposure index, stress, maternal age and deprivation, and sex ratio significance. Potential interactive processes on sex ratio alteration are therefore modelled, such as pollution and deprivation, and pollution and maternal age. Additionally, spatial statistical techniques in ArcGIS are used to produce an Inverse Distance Weighting map of birth sex ratio in Scotland.

4.5 Methods: Principles and Advantages

Some of the methods and techniques adopted in the 3 stage analysis are described in further detail in the following section. Time-series analysis, linear regression, bi-variate correlation and the advantages of stratification are discussed. Further, the principles of exposure assessment and GIS best practice are commented on, including their relative strengths for environmental and social epidemiological inquiry. A brief discussion of the case study approach is given with justification for the designating Central Scotland as the suitable region for investigation.

4.5.1 Statistical Analysis: Time-series, Regression, Correlation and Stratification

Time-series projections and analysis are undertaken in many of the sex ratio studies discussed in Chapters 2 & 3, ranging from simple tabulation of male and female births over 5 year time periods to Box-Jenkins auto-correlation tests of linear time trends e.g. sex ratio and national economy indices. The principle of time series analysis is that by collecting univariate data with values recorded at successive intervals, patterns of change can emerge which may be elucidatory. Detection of trends is most likely to be successful when measurement is over a sufficiently long time period over equally spaced periods. For sex ratio trends in this thesis annual equispaced observations are displayed.

Simple linear regression is a statistical method which can be used for establishing whether significant changes have occurred over time. It estimates the relationship between a dependent variable and explanatory variables. A helpful way of displaying such a relationship is through scatterplots with the dependent variable on the Y vertical axis (in this case, sex ratio) and independent variable on the Y axis (time in years). A straight line relationship can also be plotted using the regression calculation of *ordinary least squares*. This involves the minimisation of the sum of squared deviations from the line to each plotted point (i.e. sex ratio value to year) above and below it. Regression is frequently applied to analyse multiple independent variables with many advanced modelling techniques adopted. Its straightforward application in this thesis, however, is to test for significant alterations in sex ratio over time.

Generally, before embarking on regression analysis, statisticians test to see *if* there is a relationship between two variables i.e. correlation. Importantly, as discussed in 2.1.4., a correlation does *not* indicate that one variable causes another. It can be applied using Pearson's correlation co-efficient and Spearmans rho, the former used for continuous data and the latter for ordinal values, or continuous values converted to rankings. Scatterplots can also be designed to display the data, permitting a figurative representation as to whether variables are positively or negatively associated, or randomly distributed.

The final statistical technique adopted is that of stratified analysis, or stratification. This involves grouping data into categories or ranges of values. It is often used where data can be irregular or randomised and associations are unlikely given the pattern of the data. Sex ratios by geographical areas, measured in this thesis, are data which would all into this category. It is also beneficial for displaying such data in a spatial mapping format.

4.5.2 Exposure Assessment

Exposure assessment, although difficult to obtain, is a method which is a major factor to the reliability and validity of findings in much of environmental health. Exposure is a phenomenon which involves any contact between a substance or chemical in an environmental medium (water, soil or air) and the surface of the human body, such as the skin or respiratory tract (Nieuwenhuijsen 2003). Assessment of exposure involves studying the distribution and determinants of substances or factors which affect human health (Nieuwenhuijsen 2003).

An important component of any environmental epidemiological study is also trying to define and examine the exposure pathway. This requires establishing the physical course which a substance takes to reach and interact with the human body. It also means accounting for the dose of a chemical as well as the duration and frequency of intake. Such detailed knowledge is beyond the realms of this broader spatio-temporal investigation, though the principles involved should be accounted for in drawing conclusions from results. New toxicological concepts of teratogenic and multiple exposures, discussed in 3.4, including low dose exposures are particularly pertinent for exposure assessment in this research. This is because delayed deleterious reproductive effects beyond sex ratio alteration are potentially linked to the environmental endocrine hypothesis. The *timing* of exposure premise is therefore an important consideration for discussion in later chapters.

Speculation on exposure pathways for secondary sex ratio alteration is included in Chapter 2 on the narrative review on mechanisms and discussion from the literature review. For example, impaired spermatogenesis and/or DNA sperm damage from toxic chemicals could be responsible for male birth proportion changes in populations. For this research detecting unusual patterns in sex ratio for particular localities or regions may equate to establishing local 'sentinel health events' which can provide early detection of detrimental effects on male reproductive health. Young men may be particularly susceptible to deterioration in sperm quality, as is reported in a Czech study on seasonal air pollution using forensic spermatozoa assessment (Rubes at al 2005). The Mocarellli et al Seveso sex ratio study includes findings which substantiate similar exposure risks from dioxin. Biological mechanisms and exposure pathways associated with female reproductive health and chemical exposure are not as developed and not unexpected given the invasive nature of procedures required.

Frequently in epidemiological studies, exposure surrogates are commonly used as proxy measurement for environmental transference of chemical agents to humans. Examples include occupational categories or job titles, and grading of pollution sites, such as proximity to landfills or corporate declarations of pollutant releases. Estimates and assumptions are often made by researchers in establishing surrogates and thus studies are frequently open to methodological critique. Surrogate exposure measurement, in the form of point source endocrine disruptor air pollution modelling, is included in this study. Unique to this research, however, is also the inclusion of health-related and socio-economic indicators, such as deprivation and age, which in addition to pollution, contribute to cumulative and multiple exposure pathways.

4.5.3 Spatial Analysis: Geographical Information Systems

A valuable tool for a sophisticated assessment of exposure can incorporate Geographical Information Systems (GIS). The need for further sex ratio research which applies GIS to any analysis was a finding of the previous chapter's review. GIS, a computerised spatial analytical technique, can store enormous amounts of data, construct digital maps and perform comprehensive geographical and statistical analyses. It is a tool that has been widely adopted in public health and epidemiological inquiry, particularly for health service planning and disease surveillance. A key attribute of any geographical study is the ability to conceptualise phenomena across <u>both</u> space and time, thus offering the advantage of viewing and analysing evidence across different spatial scales, as well as varying time periods (Gatrell & Elliott 2009). The multi-factorial and temporal nature of sex ratio determination and change, together with the large datasets available in Scotland, provide a highly suitable basis for a geographical study and the use of GIS tools. GIS can initially serve as an effective descriptive function for displaying maps of sex ratios, and then be applied as an analytical tool for geo-statistical inquiry.

With the growth of GIS, more geo-statistical techniques have been developed, including spatial scan statistics, geographically weighted regression, kriging, nearer neighbour analysis, quadrat analysis and multidimensional scaling (Moore & Carpenter 1999).Viel, Floret & Mauny's (2010) inquiry using cluster detection tests and spatial scan statistics, is the only published analytical GIS study examining potential effects on secondary sex ratio. Of importance also is Williams, Lawson & Lloyd's (1992) widely cited local environmental epidemiological study, which uses quantitative geography and smoothing techniques before advanced GIS tools were available.

When considering which analytical tool or tools to adopt and designing the spatial analysis, including the geography and data, there are important methodological issues to consider. Firstly, as Tobler's law of geography states, spatial data are not randomly distributed and therefore the degree of spatial auto-correlation, which can impede standard statistical tests, should be assessed (Maantay 2010; Moore & Carpenter 1999). Secondly, GIS software packages were originally designed for use in geology and remote-sensing and therefore spatial-temporal analytical tools are not well developed (Jacquez 2000). Given this deficiency, the extent to which sex ratio changes over time examined in the current research also requires methodological consideration. Thirdly, different spatial resolutions applied can produce very different results, so the choice of geographical scale(s) for any research project is crucial (Buzzelli 2005; Moore & Carpenter 1999b; Sheppard et al. 1999).

For this study, inverse distance weighting (IDW) technique and simple overlays are applied. The former involved plotting the 6,505 population centroid points for each datazone in Scotland. This geographical data-set is sourced from SNS and are representations of the centre of population gravity, as opposed to the geometric centres of datazones. The methods for their construction involved calculation of 'average' grid references for postcodes, weighting these postcodes by household count and then selecting the grid reference closest to the average of all postcodes. IDW mapping is produced using raster digitising, the graphic representation of cells (or pixilation) to construct spatial data. For this technique, the raster cell values are computed by their inverse distance from the population centroid points and the sex ratio ascribed to these points i.e. datazone sex ratios 2001-2010. The advantage of the final IDW map is the display of secondary sex ratio across a whole geographical surface rather than ‘compartmentalisation’ of the statistic to designated regions or zones.

4.5.4 Case Study: Central Scotland

Although GIS is an exceptionally powerful tool for handling large amounts of data, there are still time and resource constraints associated with data processing of pollution measurements and their geographical projection. Spatial analysis is therefore performed at a sub-national area, or case study, which includes 1620 datazones. Meaningful findings can also arise from focusing on a regional area with particular localised environmental and/or socio-economic determinants rather than a broader, more generic national study. A spatial analysis of 6 local authority areas within Central Scotland, including Falkirk, Fife, Stirling, North Lanarkshire and South Lanarkshire and Clackmannanshire follows. Although this is not a formal region in administrative terms, for the purposes of this research it is defined as *Central Scotland*.

Central Scotland is a suitable case study firstly because of the ubiquity of pollution within some parts of the region. This particularly relates to air quality in Falkirk and Fife and an industrial legacy of land contamination across the Central Belt including Lanarkshire. Secondly, there is a reasonable diversity in socio-economic and health status across the region. Thirdly, three of the local authority areas, Stirling, Fife and South Lanarkshire have large rural hinterlands which provide useful contrasts in physical environments and urban and rural populations. Finally, a census survey on household mobility within Scotland shows that areas within this region are some of the least mobile in the country (General Register Office (Scotland) 2007).

4.6 Data Quality

This section comments on the validity and reliability and quality of the secondary data to be examined. As well as data for the dependent variable, there are 9 indicators or variables in total to be included in the analysis of the Central Scotland region, which are derived from 4 government indices and databases. Some indicators are listed in more than just one of the government sources and for the analysis indicators are sometimes grouped together as composite measurements.

4.6.1 Birth Data (Sex Ratio and Maternal Age)

The Scottish Neighbourhood Statistics dataset serves as an 'umbrella' source for all population data for small areas in Scotland, with the Scottish Government using such information to develop targeted policies in very specific localities. It covers socio-economic, population and environmental data derived from a wide range of agencies and surveys. For the calculation of birth sex ratios, the totals of live male and female births per year are sourced from GRO (Scotland). This can be accessed as a web-portal and is the primary source, in this study. Maternal home addresses of recorded births are geo-coded to census areas and male birth proportions can then be calculated for different geographical units. These administrative zones/regions are described in Table 4 below, showing average population size and the compatible larger areas within which they are nested.

Table 4: Scottish Neighbourhood Statistics Geography

Area Name **/ Years**	**Total Number**	**Average Size or Range (approx.)**	**Nested In**
Data Zone 2001 - 2011	6505	800 residents	Intermediate Zone
Intermediate Zone 2001 - 2011	1235	4,200 residents	Local Authority
Local Authority 1991 - 2011	32	16,000 (Shetland) to 445,000 (Glasgow) electorate	Scotland, occasional overlap in Health Regions
Health Board Region 1973 - 2011	14	26,000 persons (Western Isles) to 1,200,000 persons (Greater Glasgow)	Scotland
Electoral Ward 1999 - 2008	1222	4,100 residents	Local Authority
Scottish Parliamentary Constituency 1999 - 2007	73	55,000 electorate	Scotland

(Adapted from: Scottish Neighbourhood Statistics, http://www.sns.gov.uk/Data Guide, 2010 & Boundary Commission for Scotland, http://www.bcomm-scotland.gov.uk/)

For this study the primary geographical resolutions for presenting results is through datazones, local authorities and health board regions. Datazones are the smallest administrative areas from which male birth proportion can be calculated and were instituted in 2001, based on that year's census output divisions. The agglomeration of these census output areas (approximately 120 residents) to datazones were undertaken to respect physical boundaries, have compact shapes and contain households with similar characteristics. There has however been criticism by individual local authorities of some of these boundaries according to such criteria, being described as 'illogical' for some localities (Scottish Government 2011). Despite this, datazone geography has remained consistent since 2001, however adjustments are likely to be made in the future given urban expansion and changes in population characteristics for some areas.

The 32 local authority areas in Scotland is the unitary structure for local government introduced in 1996 following a re-organisation of boundaries and removal of regional councils. The data used for births however, extends from 1991 up to 2010, thus providing consistent geography with significant longevity. Heath Board Region geography meanwhile is advantageous to validity of this study, with consistent boundaries maintained since 1973, the most extended time period of all areas. As the regions were instituted at the time for the old local government structure there is not exact 'nesting' of current local authorities within Health Boards. Some regions maintain integrity in nested geography with for example, the Forth Valley Health Board Region covering the local authority areas of Clackmannanshire, Falkirk and Stirling. An additional zoning included in Stage 1 of the analysis are Scottish Parliamentary Constituency areas which generally delineate easily identifiable towns and city areas and contain population numbers between that of datazones and local authority areas. Although, a possible alternative measurement of sex ratio in small areas, council wards are not suitable for the analysis. Boundaries have not remained consistent in many areas and a lot of metropolitan wards changed to larger 'multi-member wards' in 2008.

There are also a number of considerations with regard to the quality of this birth data. Firstly, it is impossible to eliminate a degree of error in the final statistics given the multiple data transfer involved, from parent, to local registrar to GRO (Scotland). There may be occasional inaccuracy with coding the maternal address to a particular datazone, though errors with simple declarations of the sex of the newborn are highly unlikely. Secondly, in terms of validity, this data set inevitably excludes some male exposures, as fathers of the births recorded may not be domiciled in the same data zone as the maternal home address. Overall, however the accuracy of birth sex data and it's geo-coding in Scotland is not compromised.

The *'percentage of first-time mothers aged 35 an over'* (maternal age indicator) is sourced from SNS which in turn has been extrapolated from Information Services Division - NHS Scotland maternity records. The SMR02 form is completed following a mother and child's discharge from hospital, which records birth size, ante-natal smoking status as well as mother's age. There is slight discrepancy however, between the hospital recorded data of ISD from SMR02 forms and GRO (Scotland) registrations and data which include every birth event in Scotland, including home births. It is estimated there is 97% to 98% coverage in the ISD maternity and birth data compiled in SNS with all births in Scotland compared with 100% inclusion for GRO (Scotland). It would be reasonable to assume though that for this specific maternal age indicator virtually all births are likely to be at hospital given the greater likelihood of obstetric complications for first-time pregnancies.

A key validity concern for this indicator is the measurement of only first-time mothers. A direct testing of association with advanced maternal age and sex ratio cannot be made given multiple parity females are not included. The utility of the indicator is thus based on the spatial concentration of older first-time mothers in Scotland thus reflecting a population structure of advanced parental age in particular areas. On assessment of previous parental cohort/cross-sectional birth studies maternal age is a reasonable proxy indicator of combined parental age (de La Rochebrochard and Thonneau 2002). Further, paternal age at birth figures is not available at small-area level in Scotland though does exist by request from GRO (Scotland) on a national, historical basis.

4.6.2 Socio-Economic Status: Scottish Index of Multiple Deprivation

The Scottish Index of Multiple Deprivation (SIMD) is a measure of small area concentrations of multiple deprivation across Scotland, and translates into the socio-economic indicator for this study. It is measured for datazone geography (as above, Table 3) and is tabulated in Scottish Neighbourhood Statistics as well as being displayed in digital map format on the SIMD web portal.

Deprivation is a well-established term and measurement in social scientific academic and policy work, reflecting a person’s or family’s lack of resources which result in poverty or financial hardship. Multiple deprivation expands this definition to include not only income deprivation but all other forms of deprivation, such as education, employment, access to services and crime. All of these elements constitute multiple deprivation and contribute to poverty in society (Scottish Government 2009;Townsend, Phillimore, & Beattie 1988). SIMD includes 7 domains which measure these separate forms of deprivation which are shown in Table 4, together with the specific government data and indicators used. The SIMD is presented as a ranking of 6,505 datazones and 20 categorical vigintile divisions rather than as continuous data.

4.6.2.1 SIMD: Area Measurement and Factor Analysis

These data are collected by locational indexing of census areas which are aggregated to datazones. Deprivation indexes in the U.K. are *area-based* measurements which identify geographical concentrations of deprivation indicators, as opposed to the aggregate of individuals who are deprived and experiencing poverty within a neighbourhood or wider area. The accuracy of such a measurement can therefore be questioned, as a large number of deprived people can live in non-deprived areas, and people not experiencing poverty can also be residing in SIMD zones. (Bailey et al. 2011; Noble et al. 2006).

Table 5 represents the most recent construction of SIMD, illustrating its composite nature and the large number of measurements calibrated. Criticisms of multiple deprivation index methodology have been directed at all previous UK indexes and these remain relevant to the current Scottish edition.

Firstly, factor analysis is used to incorporate some of these indicators into the index which adds problems of unreliability. Some of the measurements detailed in Table 4 can be sensitive to error, and factor analysing can amplify inaccuracy by using a ‘shrinking’ technique to standardise the measures. (McConnachie & Weir 2005). Secondly, data on some indicators, such as health, are collected *between* censuses and although this can improve the range of measurement covered, the use of such national survey data estimated to small areas can be prone to error (Social Disadvantage Research Centre 2002). Finally, in the Employment Domain of Table 2, there is also evidence of double counting, with Incapacity Benefit and Severe Disablement Allowance both totalled.

Table 5: Scottish Index of Multiple Deprivation Domains and Measurements

Deprivation Domain	**Measurement / Indicators**
Employment	• *Unemployment Claimant Count (average over year)* • *Incapacity Benefits recipients* • *Severe Disablement Allowance recipients* • *Compulsory New Deal participants*
Income	*Adults and children in:* • *Income Support households* • *Pension Credit households* • *Job Seekers Allowance households* • *Tax Benefit families*
Crime	*Recorded crimes rates for:* • *Domestic house breaking* • *Drug offences* • *Minor assault* • *Crimes of violence* • *Vandalism*
Housing	*Persons in households which are:* • *Over-crowded* • *Without Central Heating*
Health	*Standardised Mortality Ratio* • *Comparative Illness Factor* • *Emergency admissions to hospital* • *Proportion of population being prescribed drugs for anxiety, depression or psychosis* • *Proportion of live singleton births of low birth weight* *Hospital Episodes related to:* • *Drug Use* • *Anxiety, depression or psychosis*
Education	• *School pupil absences* • *Pupil performance at Stage 4 SQA* • *Working age with no qualifications* • *17-21 year olds enrolling in F/T higher education* • *School leavers not in training, education or employment*
Access	*Drive times to:* • *GP, shopping centre, petrol station, primary and secondary schools* • *Public transport times to;* • *GP, shopping centre, post office*

(Adapted from: Scottish Government, Scottish Index of Multiple Deprivation 2009)

4.6.2.2 SIMD: Weighting

The process of weighting is a key technique in constructing the index, and the scores and equivalent percentages for each domain are shown below:

Table 6: SIMD Weighting

Deprivation Domain	Employment	Income	Education	Housing	Crime	Health	Access
Score/ Weighting	12	12	6	1	2	6	4
Percentage	28.00%	28.00%	14.00%	2.00%	4.00%	14.00%	8.00%

(Adapted from: Scottish Government, Scottish Index of Multiple Deprivation 2009)

A key controversy for the use of SIMD is the lack of clarification as to how these weightings are set. The assignment of these values is somewhat arbitrary as although 'theoretical considerations' are chosen as the weighting criteria, there is no justification as to how these are formulated to derive the scores (Deas et al. 2003). Furthermore, the argument of political neutrality in constructing the weightings is questionable. Decisions on the significance of economic, social and environmental factors in determining poverty are, in themselves, value judgements. Any attempt to quantify the social construct of socio-economic status though is subject to criticism from a critical sociological geographical perspective.

A review of the English Index of Multiple Derivation meanwhile concludes that weighting also *'systematically downplays the extent and intensity of deprivation in major urban centres'* (Deas, Robson, Wong, & Bradford 2003). This is due to lesser uptake of benefits and greater informal economies in cities not being reflected in a measure of income deprivation. Further, the combination of including the crude measurement of road distance for access, and the relative greater weighting for the access domain, over-emphasises rural deprivation at the expense of urban (Deas, Robson, Wong, & Bradford 2003). This bias is magnified even further in the Scottish index given the characteristics of Scotland's dispersed rural settlement and urban concentration. A poor physical environment is also downplayed in the index thus skewing higher deprivation scores to rural areas, as urban-centric measures such as air quality and derelict land are not included as indicators.

4.6.2.3 SIMD: Data Quality and Data Formats

Methodological limitations of the SIMD are therefore numerous and not surprising given the composite nature of the index and the need to "equalise" data from multiple sources. Such weaknesses perhaps ostensibly relate to deprivation indexes being principally policy driven and serving as tools for resource allocation by governments in urban regeneration and planning. Alternative methods of measuring deprivation and/or socio-economic status, such as through the British Household Panel Survey and/or Scottish Household Survey, or by quantifying Bourdieu's concepts of social, economic and cultural capital, have been suggested as alternative or complementary indicators (Tomlinson, Walker, & Willaims 2011). The deprivation area-based indexes, however, have proved popular for epidemiological research in particular, such as for life-course and chronic diseases studies. Further, identification of the area-based or neighbourhood effect on poverty, whereby being poor in a deprived area compounds disadvantage, also validates the index as a measure of socio-economic status (Atkinson & Kintrea 2001; Pacione 1995). Critical geography scholars have emphasised the growth of a 'cottage industry' in academia on neighbourhood and area effects of poverty and health whereas the structural implications of neo-liberal economics on employment and housing in determining area-based outcomes are frequently overlooked (Slater 2013).

The Scottish Index of Multiple Deprivation contains inevitable weaknesses in validity and reliability given its challenging methodological ambition of quantifying poverty by area in populations. Not least of these is the area-based or ecological fallacy problem, of not detecting deprived people in non-deprived areas, and ascribing collective characteristics to all individuals residing in an area. However, SIMD and other deprivation indices have undergone continuous refinements for improving validity and reliability. Further, social epidemiological studies which utilise deprivation measurement overwhelmingly reinforce the validity of the index by demonstrating area-based poverty and poor health outcomes. On understanding its limitations, SIMD though offers a valuable and improved measurement of socio-economic status compared with many previous sex ratio studies. The conspicuous conceptual framing of multiple deprivation as spatially and environmentally determined also concords with similar disciplinary perspectives of environmental health and socio epidemiology pivotal to this study.

4.6.3 Health Status (Smoking and Neighbourhood Stress): Scottish Public Health Observatory

Data for maternal smoking and neighbourhood stress are sourced from Community Health Profiles located in the Scottish Public Health Observatory (SPHO). The SPHO is an information portal/service developed by NHS Health Scotland and the Information Services Division (ISD) to support health improvement and decision-making in Scotland. There is an overlap of this public health data with Scottish Neighbourhood Statistics and the Scottish Index of Multiple Deprivation. The Information Services Division is the official health statistical agency in Scotland which collates and produces the original NHS source data. SPHO published Community Health Profiles (CHP) for 2008 and 2010 of local authorities and community health partnerships, including measurement of indicators within data zones. These comprise 61 key health indicators, both on mortality and injury as well as for determinants of health, such as deprivation and employment status. Three of these indicators, listed in Table 6, can be included as variables in this research; each corresponding to factors emerging from the scoping review. The metadata attached to this information are also outlined, permitting an assessment of their accuracy. Two of these indicators, anxiety prescriptions and alcohol hospital admission, form the proxy indicator of neighbourhood population stress, which also includes SIMD measurement of local crime rates.

4.6.3.1 Smoking at Ante-Natal Booking

The smoking at ante-natal booking indicator is a measurement of the percentage of pregnant women which report whether they are smokers at their first ante-natal clinic. Although levels of smoking are not established, the specificity of the indicator to maternal health and research hypothesis on smoking warrants its inclusion in modelling and spatial analysis. There are, however, concerns over inaccuracy, with gaps in early recording occurring in 1993/94 and 'more recently' according to ISD. (Scottish Public Health Observatory 2011). Empirical research also concludes that self-reporting in ante-natal settings under-estimates the true level of maternal smoking (Ford et al. 1997; Shipton et al. 2009). For populations where smoking incidence is low the self-reported inaccuracy is relatively small (Klebanoff et al. 2001) . However, a recent West of Scotland study reports a 39% underestimate in areas where smoking is far more prevalent, more specifically in deprived communities (Shipton, Tappin, Vadiveloo, Crossley, Aitken, & Chalmers 2009). The investigation reports that up to twice as many smokers go undetected in deprived areas compared to non-deprived. Such under-reporting of maternal smoking emphasises the importance of investigating localities of health deprivation, particularly given results from previous studies of smoking and sex ratio alteration, such as the Liverpool study.

Table 7: Scottish Public Health Observatory: Community Health Profile Indicators

Indicator	**Secondary Sex Ratio Linked Factor**	**Metadata**
Percentage of women smoking at ante-natal booking	Maternal Smoking	Self-reported information obtained from mothers at ante-natal meeting in community or hospital
Estimated number of people being prescribed drugs for anxiety, depression or psychosis	Population Stress	Estimated patients being prescribed based on applying GP practice average prescribing rates to small-area populations. Average daily quantity (WHO standards) used to calculate from, weight of total prescriptions at practice, an average count of people being prescribed.
Number of patients hospitalised with alcohol conditions	Population Stress	2008: Number of admissions to acute and psychiatric hospitals in Scotland with a main or secondary diagnosis of alcohol related conditions. 2010: Patients discharged from hospital with at least one Patient Attributable Fraction for alcohol (includes 53 health conditions). Psychiatric hospitals not included.

(Adapted from: Scottish Public Health Observatory - http://www.scotpho.org.uk/home/home.asp)

4.6.3.2 Proxy Indicators for Neighbourhood Stress

Population stress and birth sex ratio adjustment examined in previous research concerns natural disasters or acute incidences of public violence. There is also, however, substantive research on associations between psycho-social stress and peri-natal effects in urban neighbourhoods, with poverty, crime and poor health cited as factors (Elo et al.

2008; Farley et al. 2006). A combination of proxy indicators for population stress in Scotland linked to neighbourhood effects and medication uptake in response to stress can be applied using SPHO indicators. These include persons prescribed anti-depressant and anti-anxiety drugs, alcohol admissions to hospital, and SIMD crime measurement.

The indicator for anxiety is the estimated number of people being prescribed drugs for anxiety, depression or psychosis within a datazone. This statistic is constructed on the basis of total weight of prescriptions, as publishing the numbers of patients prescribed runs the risk of disclosure within such small areas. The estimation figure, which also uses average prescribing rates, is therefore not entirely reliable. It is also not clear whether all patients attending these GP practices live within the designated datazone ascribed. Despite these shortcomings, the prescription figures may give reasonable and valid estimates of levels of anxiety within small areas, and also potentially avoid multi-collinearity issues with other deprivation indicators.

Alcohol-related hospital admissions can also serve as a partial proxy indicator for population stress, both as a symptom of stress itself and as a causal factor. As the measure is a count of persons attending hospitals, and their usual residence is recorded on discharge, the indicator can be judged as reliable. The omission of psychiatric hospital admissions in 2010 though, does mean some slight inconsistency of measurement between years.

The final proxy indicator for neighbourhood stress is recorded SIMD crime rate, included in the Community Health profiles and sourced from SIMD. As listed in Table 5, specific crimes measured are domestic housebreaking, minor assault, drug offences, crimes of violence and vandalism. Incorporation of SIMD crime rates as a proxy stress indicator replicates analysis undertaken in U.S. cities on peri-natal stress and neighbourhood crime (Messer et al. 2006). A limitation of such measurement is the highly mobile nature of some crime, such as gang-related violence and offences linked to events (e.g. football matches) specific to particular localities and times. There are also likely, as a result of disclosure control, to be occasional calculation errors when extrapolating from small counts in some areas.

A final methodological issue attached to both maternal smoking and neighbourhood stress data is strong spatial auto-correlation with multiple deprivation. Documented extensively in social epidemiology, these health determinants are substantively inter-related and multi-causally linked. This introduces the problem of multi-collinearity in any multiple regression models as variables are assumed as independent of each other. A stratified analysis of each variable - deprivation, stress and maternal smoking - may isolate specific likely causal factors although caution still needs to be exercised given the strong correlation between the variables and thus danger in making fallacious associations.

4.6.4 Endocrine Disruptor Exposure (Air Pollution): Scottish Pollutant Release Inventory

The environmental pollution mapping and variable of modelled air pollution of source endocrine disruptor emissions, is primarily constructed from SEPA's Pollutant Release Inventory. As described earlier, endocrine disrupting pollutants were identified on the SEPA inventory by cross-reference with the TEDX (Endocrine Disruptor Exchange**)** data-base. Methods for modelling point source emissions from SEPA registered emitter sites, including data sourced, are also discussed.

4.6.4.1 Scottish Pollutant Release Inventory

The Scottish Pollutant Release Inventory (SPRI) is a publicly accessible database which specifies annual mass releases, in kilograms, of pollutants to air, water and land from industrial sites which are SEPA regulated. A key advantage in using SPRI is that emissions can be searched by specific chemical or pollutant and the index displays the polluter's registered company name and site address. This enables, in the first instance, mapping of individual sites of pollutant releases together with the annual emission values.

One obvious weakness of the inventory is that there are no data for 2003. SEPA state, because ‘the (then) Scottish Executive asked SEPA to build a better, more comprehensive SPRI’ thus giving time for emission site reporters to conform to the 2004 new requirements. It is also clear from searches on the inventory that only a limited number of common air pollutants were reported (e.g. nitrogen oxide, carbon monoxide) in 2001 and 2002. A further limitation is that emission values are only declared if above particular chemical threshold levels determined by SEPA. These thresholds are prescribed based on knowledge of each pollutant’s ‘toxicity, transport and persistence in the environment.’ Recent toxicology reports and models on endocrine disrupting compounds however, show possible detrimental health effects at very low levels of exposure (Birnbaum and Fenton 2003) which are unlikely to be measured in the SPRI given their likely discharge below the thresholds set.

A further assessment of data quality concerns the methods adopted by operators for making emission declarations. SEPA's guidance to registered emitters on how to produce emission declarations is instructive. Operators can determine releases according to one of three method types; Measurement, Calculation, or Estimation. These are detailed further in the table below:

Table 8: Scottish Pollution Release Inventory Methods

Method Type	**Summary**
Measurement	• Based on short-term and spot measurements • From direct monitoring results for specific processes at a facility
Calculation	• Uses activity data e.g. fuel used, production rate • Complicated methods include variables such as temperature and global appliance
Estimation	• Best assumptions or expert guesses • Description of methodology must be reported to SEPA

(Adapted from SEPA website, 2011

http://www.sepa.org.uk/air/process_industry_regulation/pollutant_release_inventory/methods_and_ methodologies.aspx)

SEPA make comment on the efficacy and feasibility of each of these methods. *Measurement*, in the form of direct monitoring is an ideal measure for specific substances, although it is recognised by SEPA as highly impractical in cost terms. Sampling as an alternative method is suggested to be used cautiously especially when extrapolating to annual releases (Scottish Environmental Protection Authority 2011). Engineering *calculations* are also difficult to rely on because of the level of detail needed on inputs and outputs within an operation. Further to this, modelling on site can differ from the methods used to develop the original source model. Finally, *estimation* and engineering judgement is the least costly, relying on intuition and taking advantage of experience and professional knowledge (Scottish Environmental Protection Authority 2011).

Examination of SPRI results tends to show a pattern for these method types, with measurement used for water emissions, calculations for common air pollutants and estimations for earlier recordings and less common air pollutants. Although these method types are declared there is little detail on how each is adopted, such as whether sampling is used or how ‘best assumptions’ are made. Given the wide, flexible criteria for determining pollutant releases for operators the inventory is likely to be subject to some inaccuracy. This may be particularly the case where estimation is chosen as the method type by operators and there is a reliance only on *intuition* as opposed to scientific method.

Academic research on environmental disclosure and Pollutant Release inventories has been conducted in the U.S.A. and Australia, and findings suggest under-estimation by companies of 'true' levels of pollution (Lyon & Maxwell 2011). There are currently no similar assessments in the academic literature of the effectiveness of SPRI for measuring pollution in Scotland. However, a recent press article from the Sunday Herald reported serious errors of over-estimation in the SPRI, including for the potentially harmful chemicals of dioxin, mercury and cadmium releases (Edwards 2011). There are some concerns then as to the accuracy of SPRI, though a data-set offering site-specific measurements or estimates of source pollution can assist in distinguishing low and high levels of potential exposure for different areas or regions.

4.6.4.2 The Endocrine Disruptor Exchange (TEDX)

Identifying air pollution specific to potential endocrine disruption was undertaken by cross-referencing the SPRI pollutant database with a list of over 850 endocrine disrupting compounds documented by the Endocrine Disruptor Exchange Inc. (TEDX). This is an incorporated non-governmental organisation which exclusively focuses on the environmental and health effects of endocrine disruption and disseminates and compiles relevant research. It is founded by Theo Colborn a key organiser and signatory to the original Wingspread Consensus statement on 'chemically induced alterations in sexual development'. The TEDX List of Potential Endocrine Disruptors is a database of chemicals with the potential to affect the endocrine system. Every chemical on the list has one or more verified citations of a scientific paper reporting potential hormone disrupting properties. The chemical listing also includes CAS numbers thus enabling easy cross referencing with the pollutant inventory.

4.7 Air Pollution Modelling: Energy for Waste Facilities - Environmental Statements

Simplified modelling is undertaken by approximating from Air Quality predictions available from Environmental Statements on locally proposed Energy for Waste facilities. Met Office wind roses are also utilised to estimate the air pollution models for Central Scotland. This approach was adopted because of specific difficulties with other sources and methods.

There is no publicly available data on the dispersal and distribution of generic endocrine disruptor air pollution in Scotland. SEPA responded to enquires made about such modelling that it has not been undertaken ‘on a large scale’ and is ‘so far focussed on some individual processes’ (Scottish Environmental Protection Authority 2011). For the Grangemouth area, SEPA suggested results from Falkirk Council’s commissioned reports on the Air Quality Management Area (AQMA) as a source of modelling data. AQMAs are declared by local authorities in the U.K. if national air quality objectives and standards, set according to thresholds of health effects from pollution, are not being met. These principally apply to exposures around traffic congestion in urban zones however Grangemouth is the only AQMA in Scotland designated which addresses industrial emissions. Three monitoring stations measure hourly concentrations of Sulphur Dioxide, Nitrogen Dioxide and Particulate Matter which form a large part of local air quality assessment. These chemicals are not listed as endocrine disruptors and although potentially instructive as proxy measurements, there are not similar readings taken in other areas of Central Scotland. GIS techniques, such as kriging, may be useful in this regard, through developing spatial representation of pollution by monitoring station point location and interpolating exposure ‘polygons’. This however, requires an extensive and reasonably uniform network of stations which tends to exist only in very large metropolitan regions and which does not apply to the case study.

Gaussian plume distribution calculation presented another possibility for modelling endocrine disrupting air emissions. Tasks to achieve this would include field measurement of over 40 emitter stacks, sourcing emission rates from each registered emitter, and undertaking ground surveys to test distribution models calculated. Owing to time constraints of the project and its multi-disciplinary ethos such detailed environmental scientific work was not undertaken.

The final formulation of modelling for the cumulative emissions totals of endocrine disrupting pollutants from SPRI was derived from further secondary sources. Statutory Environmental Statements have been published as part of submissions by private operators to build Energy from Waste (Combined Heat and Power) Facilities in Eastern Scotland at Dunbar and Grangemouth. Air Quality Impact Assessments for the predicted emissions from each facility accompany each of the statements, including details on methods and dispersal mapping in appendices. For the Dunbar statement *both* AERMOD and ADMS 4 “new” Gaussian dispersion modelling was conducted in order to account for variable results between models. The ‘worst-case’ scenario or least conservative estimates of pollution dispersal were thus reported. These modelling software packages are commercially available and developed by the U.S. Environmental Protection Authority (AERMOD) and Cambridge Environmental Research Consultants and the UK Met Office (ADMS 4). The Grangemouth assessment utilises the ADMS package as well as estimating cumulative impacts from the existing large industrial emissions in the area.

Simplified models of air pollution from the SPRI emitter sites are generated from two maps from the AERMOD and ADMIS modelling; a Contour Plot of Predicted Mean Contributions at Dunbar EfW and Annual Mean Chromium Process Contribution for Grangemouth EfW (both shown Appendix D & E). The Dunbar EfW Statement also includes Windroses for Edinburgh and Grangemouth, and together with Met Office windroses for Western Scotland (Prestwick) these were also included in estimates of dispersal direction distances for 3 areas of Central Scotland. The final model parameters are shown below:

> ***SPRI Emitter Sites in Stirling, North Lanarkshire, South Lanarkshire***: NNE and E (30 degress to 100 degrees) 3000m, SW-WSW (230 degrees to 250 degrees) 1000 metres
>
> ***SPRI Emitter Sites in Falkirk and Clackmannanshire Local Council area*** : W to WWS (70 degrees to 130 degrees) 3000m, EEN (280 degrees to 300 degrees) 2250 metres
>
> ***SPRI Emitter Sites in Fife*** : NNW 50 degrees to 90 degrees, 3000m, and SSE 240 degrees to 260 degrees, 2000 m

By applying these parameters through *'Spatial Analyst Tools'* in ArcGIS with the 'Euclidean Distance' and 'Mosiac' commands the final polygons (converted from raster files) were created. These were then transposed onto the map of XY co-ordinates for SPRI emitter sites to display the map of Endocrine Disruptor Air Pollution Modelling and gradated according to Cumulative (2002, 2004-2010) Total Air Emissions/kg.

4.8 Limitations

Although limitations in any study are usually articulated after presenting results, there are some crucial methodological issues which warrant discussion in relation to the research context outlined so far.

Firstly, the nature of the dependent variable, secondary sex ratio, is important to understand when including it for statistical model building. Given the multi-factorial context of the secondary sex ratio and also accepting that one significant potential factor, timing of conception, is essentially randomised, male and female birth proportions in populations can oscillate widely. This is in contrast to many other health statistics, and socio-economic data, which are likely to be more predictable and associated with other independent variables. The long standing finding that low socio-economic background predicts poor health outcomes acutely and across the life course is testament to this general rule. There is therefore a tendency in previous research on sex ratio, as described in the review, to include comparisons of male birth proportion across categorical variables or standardised accounts, or over-interpreting temporal sex ratio trends without measuring sufficiently all potential causative factors. The weakness of ecological fallacy, whereby population studies are ‘over-interpreted’ in drawing characteristics or events to individual cases, is distinguishable in this regard. Exceptions to this include the large national population studies on age and health factors, particularly in the U.S. and Scandinavia, where accessible individualised medical databases permit more rigorous statistical analysis. This variability in research methods and 'deductive guesswork' of some finding possibly reflects this 'randomised tendency' and unpredictability of the sex ratio statistic.

Secondly, the 'area-based' data utilised in this research further compromise the dissociative nature of the sex ratio dependent variable. There is a trade-off between the range of potential socio-economic *and* environmental variables which can be added to a geographical analysis and the imprecise nature of area-based measurement compared to individualised data-sets. The male birth proportion values used in any statistical modelling or geo-statistical analysis are based on the grouping of male births in ascribed administrative and census areas based on maternal home address rather than delineated points showing the sex of birth or an exposure parameter of location of parents from conception to birth. Importantly though, the area based measurement is to high degree of geographical resolution i.e. datazones (as discussed in 3.6.1) and the ability to also model and map environmental pollution in such small areas possibly outweighs other methodological weaknesses.

Finally, the inclusion of such geographically based variables presents the limitation of a 'moving target' in any analysis, with the characteristic of population migration in and out of the selected areas. This limitation is allayed to some extent by not including major metropolitan areas, such as Glasgow, Edinburgh or Aberdeen, in the geo-analysis, as population mobility in cities is significantly higher. In summary, all of the attributes linked to this research, volatility of the dependent variable, area based measurement and population mobility, illustrate the difficulty in building the normal predictive models compared to many social scientific investigations.

4.9 Ethics

The socio-economic and demographic statistics are all available on publicly accessible databases and are ethically approved by the government agencies involved in collection and collation of the data. The agencies and web-portals include Scottish Neighbourhood Statistics, Scottish Public Health Observatory and Scottish Index of Multiple Deprivation. The major consideration is the minimum level of geographical abstraction at which anonymity could be maintained. For example in the case of public health data, individuals could not be identified on emergency admissions amongst a population area of 400 to 600 persons.

The Scottish Neighbourhood Statistics policy is detailed below:

'To maintain confidentiality and make sure information on a particular individual or household is not revealed, one or more of the following disclosure control methods may have been used on any Neighbourhood Statistics data following the National Statistics Code of Practice.

1. *Each geographic area has a minimum population and size*
2. *Data presented for defined hierarchical and non-overlapping geographical areas*
3. *The classification in tables may be limited*
4. *Records may be swapped before tabulation*
5. *Small cell adjustment*
6. *Rounding*
7. *Suppression of small numbers'*

(Scottish Neighbourhood Statistics (2013) *Scottish Neighbourhood Statistics - Frequently Asked Questions*, http://www.scotland.gov.uk/Topics/Statistics/sns/SNSFAQs#a28)

Clearly, as the research only involves analysis of secondary data, there are no participants to recruit. Under the Data Protection Act, nearly all information can be accessed from public bodies, however it is important to articulate the purposes for which the data acquired is to be used. SEPA produces its own statement on data re-use conditions, which is shown in Appendix C.

Chapter 5: Results

5.1 Introduction

Chapter 5 addresses the central question of birth sex ratio and potential causative factors for the observed variation in Scotland and presents the thesis results including a range of figures and tables. These are presented over 3 stages; (i) a review of temporal and regional trends in secondary sex ratio in Scotland (ii) a stratified analysis of small area-based data of birth sex ratio with: multiple deprivation, maternal age, maternal smoking, and a proxy measure of neighbourhood stress; and (iii) a GIS spatial analysis of small area birth sex ratios with modelled endocrine disruptor exposure for such areas, and combined with the deprivation and age indicators.

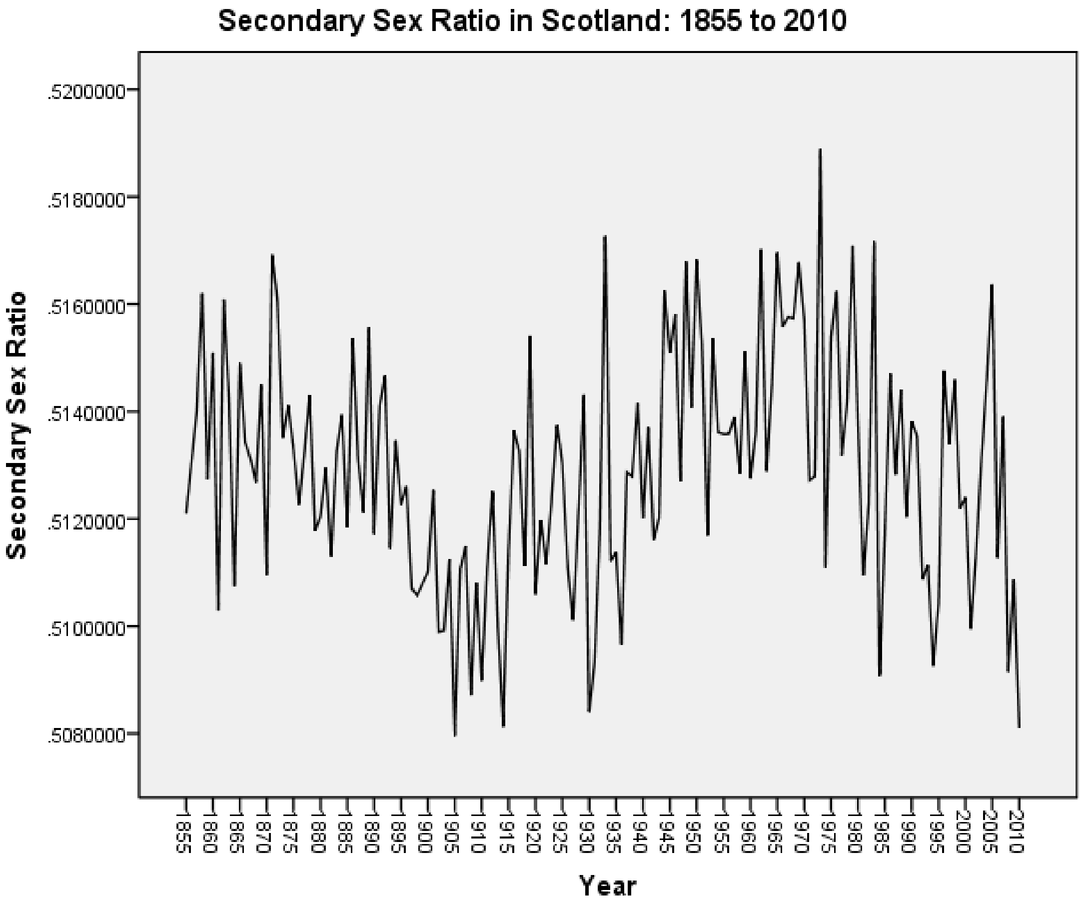
5.2 Secondary Sex Ratio Trends in Scotland

Assessment and display of temporal trends for secondary sex ratio in Scotland for national, regional and local geography are given in the first section of this chapter.

5.2.1 National Secondary Sex Ratio

Figure 3 shows the yearly historical trend of secondary sex ratio for the Scottish population from 1865 to 2008. As noted in Chapter 3, the very early birth registration system in Scotland (1865 Scotland Registration Act) permits an assessment of the male birth proportion trend for a comparatively long period. The Y axis values for this graph range from 0.508 to 0.520, reflecting a highly fluctuating sex ratio statistic between years. The lowest male birth proportion figure is 0.508 in 1905 whilst in 1973 the secondary sex ratio in Scotland reached its highest point at 0.519. Most recently in 2010, the second lowest birth sex ratio figure in Scotland of 0.508 occurred. Despite these oscillations and the sex ratio range across years it is possible to discern three important periodical trends.

Figure 3: Secondary Sex Ration in Scotland: 1855 to 2010

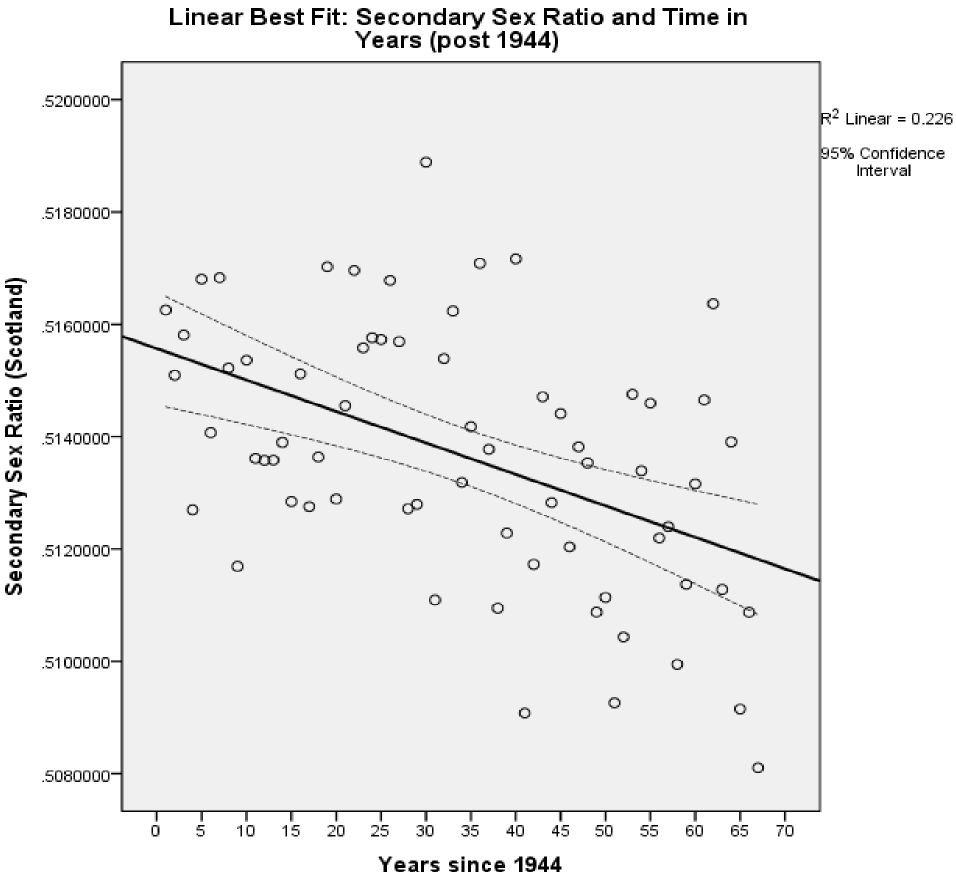


(Data source: General Register Office for Scotland 2010, accessed through www.gro-scotland.gov.uk/statistics/births/births-time-series-data.html)

The first period is from 1865 to the end of the 19th century with the secondary sex ratio showing relatively constant values of around 0.513 before dropping rapidly in the 1910-1920 decade to approximately the 0.510 level. In the second transition, a substantially long duration from the early to mid-late 20^{th} century, the sex ratio climbs to about a 0.515 peak. The third stage is the apposite period of male birth proportion decline which can be identified for Scotland. Commencement of this phenomenon is not easily discernible from the line trend, although the early 1970s represents a reasonable assessment. Further analysis of this period is undertaken in the acceding graphs and tables. The secondary sex ratio also oscillates significantly at this time, exemplified with values of 0.517 in 1993 and 0.509 in 1994, and 0.519/0.511 for 1973/1974. The 1973 peak contrasts with the 0.508 ratio drop in 2010, thus starkly illustrating the relatively *recent* decline of Scotland's birth sex ratio. The 1920-1970 increase and 1970 onwards decrease however may reflect only a very simplified analysis of trends with alternatively a plateauing of sex ratio being witnessed during post-war times up to 1972.

The recent sex ratio decline is analysed further through linear regression modelling (Figures 4, 5 and 6, Tables 9 & 10, 11 & 12, and 13 & 14). Time periods are analysed for 1944 to 2010, 1960 to 2010, and 1973 to 2010 respectively. Scattergrams with best fit graphs are provided for each period, with tables providing regression co-efficients immediately following the relevant graph. In all the graphs a negative linear relationship between sex ratio and advancing years can be observed. The largest adjusted R^2 coefficient is .227 for 1960 to 2010 (p value 0.00), whilst 0.214 (p value 0.00) is calculated for 1944 to 2010, and .146 (p value 0.010) for 1973 to 2010. The best fit model for a decrease in sex ratio over time in years is for the 1960 to 2010 period, thus suggestive of establishing 1960 as the onset of male birth proportion decline in Scotland. Despite this result though, with the 1973 to 2010 disparity in low and high national sex ratio outlined, and the slightly less best fit model of 1944 to 2010, the period of decline is not a precise estimate.

Figure 4: Linear Best Fit: Secondary Sex Ratio and Time in Years (post 1944)



Tables 9 & 10 – Linear Regression Co-efficients (1944 – 2010)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.475[a]	.226	.214	.0020353351

a. Predictors: (Constant), Year Ordinal from 1944

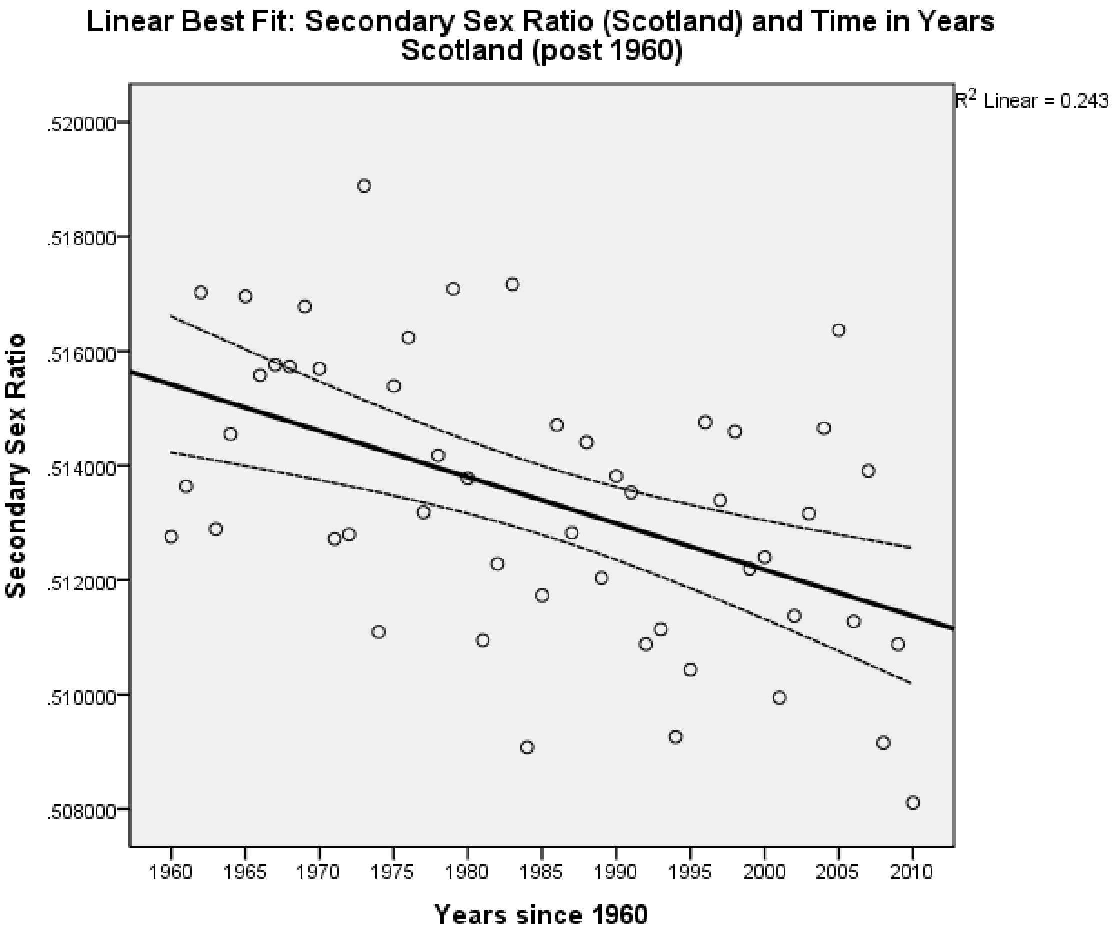
Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.516	.001		1025.126	.000
	Year Ordinal from 1944	-5.598E-5	.000	-.475	-4.354	.000

a. Dependent Variable: Secondary Sex Ratio Scotland 1944 to 2010

(Data source: General Register Office for Scotland 2010, accessed through www.gro-scotland.gov.uk/statistics/births/births-time-series-data.html

Figure 5: Linear Best Fit: Secondary Sex Ratio (Scotland) and Time in Years (post 1960)



Tables 11 & 12 – Linear Regression Co-efficients (1960 – 2010)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.493[a]	.243	.227	.002144801

a. Predictors: (Constant), Years from 1960

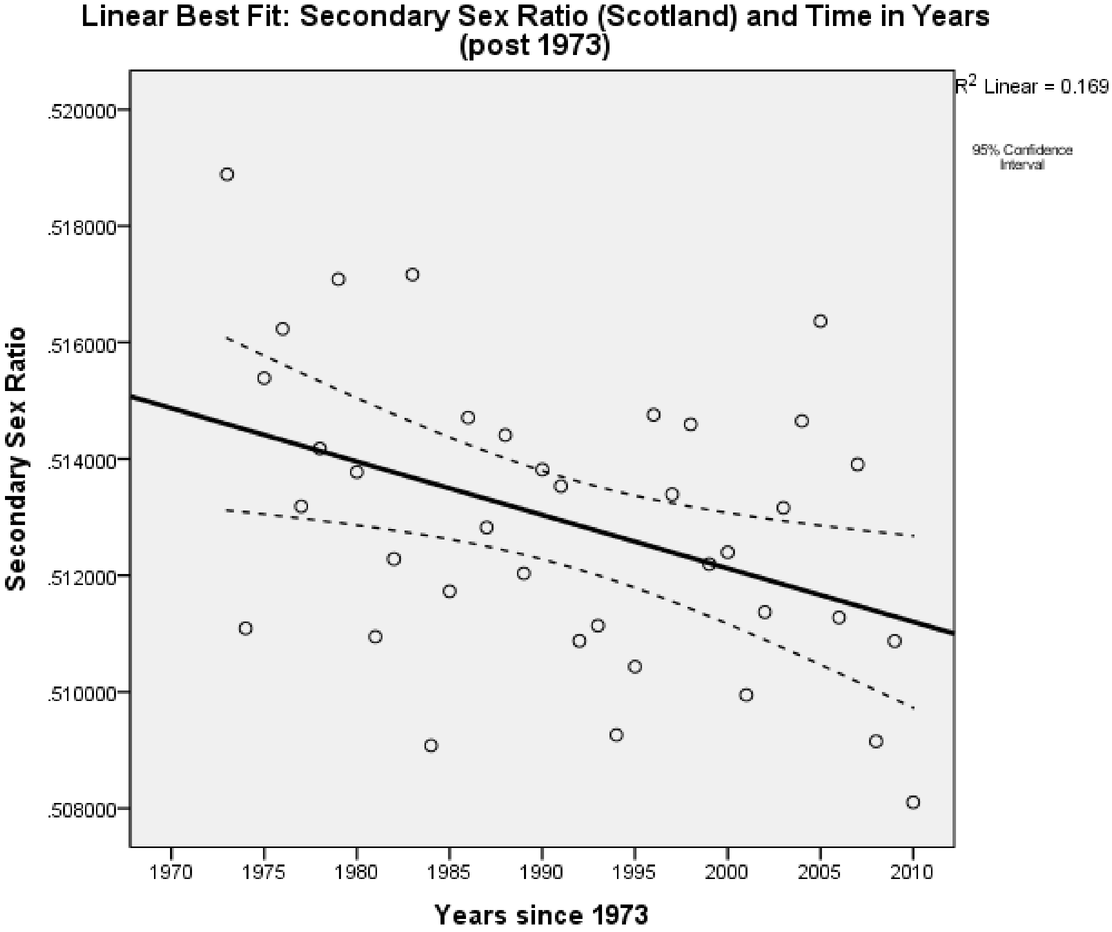
Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.515	.001		845.620	.000
	Year_ord_1960	-8.089E-5	.000	-.493	-3.965	.000

a. Dependent Variable: Sex_Ratio_1960

(Data source: General Register Office for Scotland 2010, accessed through www.gro-scotland.gov.uk/statistics/births/births-time-series-data.html

Figure 6: Linear Best Fit: Secondary Sex Ratio (Scotland) and Time in Years (post 1973)



Tables 13 & 14 – Linear Regression Co-efficients 1973-2010

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.411[a]	.169	.146	.002289588

a. Predictors: (Constant), Year_ord_1973

b. Dependent Variable: Sex_Ratio_since1973

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.515	.001		679.233	.000
	Year_ord_1973	-9.167E-5	.000	-.411	-2.706	.010

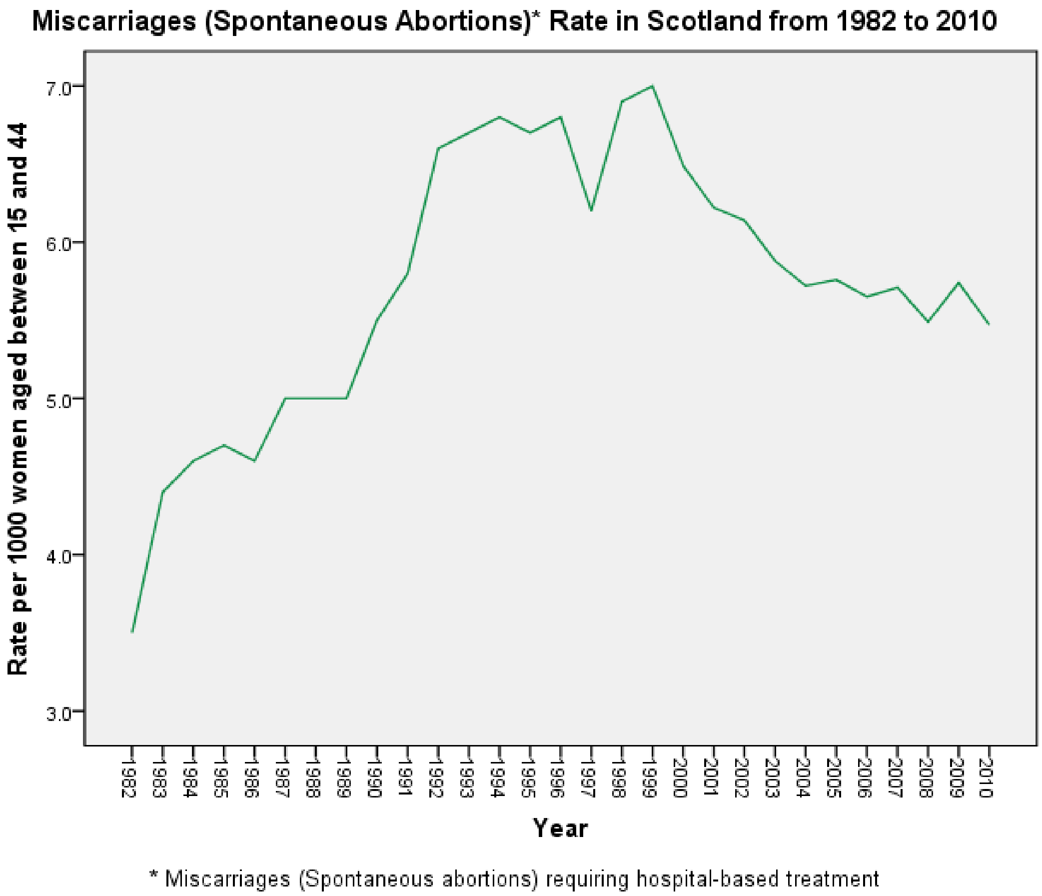
(Data source: General Register Office for Scotland 2010, accessed through www.gro-scotland.gov.uk/statistics/births/births-time-series-data.html)

5.2.2 Associated Maternal and Birth Statistics

Further relevant reproductive health trends for Scotland are also shown in Figure 7 with a linear graph for miscarriages between 1982 and 2010. The clinical coding for miscarriage or spontaneous abortion used by ISD in Figure 7 is the WHO definition; “the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age” (ISD Scotland 2009). The rates are based on NHS *recorded* miscarriages, the spontaneous abortions which required hospital-based treatment. Miscarriages which occurred at home and early pregnancy loss, where women were possibly unaware of pregnancy, are therefore not included in the statistic. Late fetal deaths, infants born dead at 20-23 weeks of pregnancy, are also not included and therefore, with the combination of these omissions, the national miscarriage indicator should be interpreted with caution. The graph does show an overall rise in the incidence of miscarriages for the first 18 years, from a rate of 3.5 in 1982 to 7.0 in 2000. After 2001 a steady decrease ensues, with 5.6 per 1000 women aged between 15 and 44 receiving hospital-based treatment for miscarriage.

Additional to these figures are important accompanying maternity and birth trends retrieved from reports produced by ISD Scotland and GRO (Scotland). Stillbirths in Scotland have decreased dramatically from the post-war period, averaging 29.2 per 1000 births between 1946 and 1950 and most recently for 2006 to 2010, down to a rate of 5.3 (GRO Scotland 2011). Historical infant mortality statistics, compiled before the compulsory registration of still births in Scotland in 1939, also illustrate these large differences, with an infant death rate of 82 per 1000 live births in 1936 (Centre for the History of Medicine 2012). All such statistics in Scotland also show the greater proportion of stillbirths and neonatal deaths affecting the male sex *over and above* the normal live birth excess of males i.e. secondary sex ratio. Figure 7 shows miscarriage (or spontaneous abortions) rates in Scotland from 1982 to 2010 with a steady rise of incidence from 3.5 per 1000 women aged 15 and 44 in 1982 to a peak of 7.0 in the year 2000. From 2000 to 2010 the rate has declined slightly (5.4 in 2010). The difficulty of interpreting from these statistics, however, is the imprecise measurement of actual miscarriages; given these are only spontaneous abortions that have required hospital treatment. This does not include a large percentage of early pregnancy loss which is either occurring at home with G.P. surgery attention or is not recognised as embryo loss by the affected female. Induced abortion rates are also recorded by ISD Scotland with increases over 20 years from 5.4 per 1000 women aged 15 to 44 in 1976 to a 10.1 rate in 1995 (ISD Scotland 1997).

Figure 7: Miscarriages (Spontaneous Abortions) Rate in Scotland 1982 to 2010



(*Data Source:* Information Services Division Maternity: NHS Scotland – requested via e-mail)

5.2.3 Urban/Rural Geography

Modelling and display of historical sex ratio trends is also undertaken based on geographical divisions created by GRO (Scotland) (see Table 15 below). Annual Reports published by GRO (Scotland) from 1946 to 1973 include statements on the national birth sex ratio as well as sex ratios calculated for the following urban/rural classifications:

Table 15: GRO (Scotland) Annual Report Geography 1946-1973

GRO (Scotland) Annual Report Geography	Description and Examples
Counties of Cities	Large urban municipalities: Glasgow, Edinburgh, Dundee, Aberdeen. *(Not a category from 1946 to 1949, Counties of Cities incorporated into Large Burghs)*
Large Burghs	Small urban municipalities and larger towns, e.g. Inverness, Clydebank, Motherwell, Falkirk *(Included Counties of Cities 1946 to 1949)*
Small Burghs	Medium to smaller sized towns, e.g. Dumfries, St Andrews, Ayr
Landward	Rural areas, agricultural districts.

A statistical summary for the above geography is given in Table 16 with mean secondary sex ratios from 1946 until 1973. The total births figures show the larger population sizes for the Counties of Cities and Landward categories whilst variation in sex ratio is negligible with only a slight deviation downwards for the Large Burgh areas. The cumulative national sex ratio from 1946 to 1973 of 0.515 is in fact greater than the previous 27 years, from 1919 to 1946, which was 0.512 (GRO Scotland figures), reflecting the gradual incline of male birth proportion over the early and mid 20th century.

Table 16: Statistical Summary for GRO Annual Reports Geography 1946 -1973

Geography	Minimum	Maximum	Mean	Total Births (N)	Std. Deviation
Scotland	.51169	.51889	.5147446	2684914	.00176723
Counties of Cities	.51018	.51932	.5152303	976527 (est.) *	.00266388
Large Burghs	.50703	.52141	.5136517	478856 (est.) *	.00400626
Small Burghs	.50887	.52100	.5150134	456678	.00394526
Landward	.50822	.52239	.5148293	772853	.00350440

*Estimated numbers of births for Counties and Cities and Large Burghs are based on 1950 total births for each category.

(*Data source*: General Register Office for Scotland, Annual Reports 1946 to 1973, Accessed at Ladywell House, Ladywell Road, Edinburgh)

The linear trends for post-war urban/rural geography are also displayed in Figures 8 & 9 with the overall sex ratio for Scotland also plotted. The only discernible pattern is an upwards movement of sex ratio for Landward areas whilst the other categories oscillate over and under the national line. The reasonably constant national linear trend observed for the 1946 to 1973 period however may not necessarily be instructive for showing declining birth sex ratio for urban geography. These data however, are the only measurements of sex ratio at the sub-national geographical level for post-war Scotland. Further insight could be gained from extrapolating beyond 1973 and delineating cities and towns into the Annual Report categories.

Figure 8: Secondary Sex Ratio: Counties of Cities & Landward 1946 to 1973

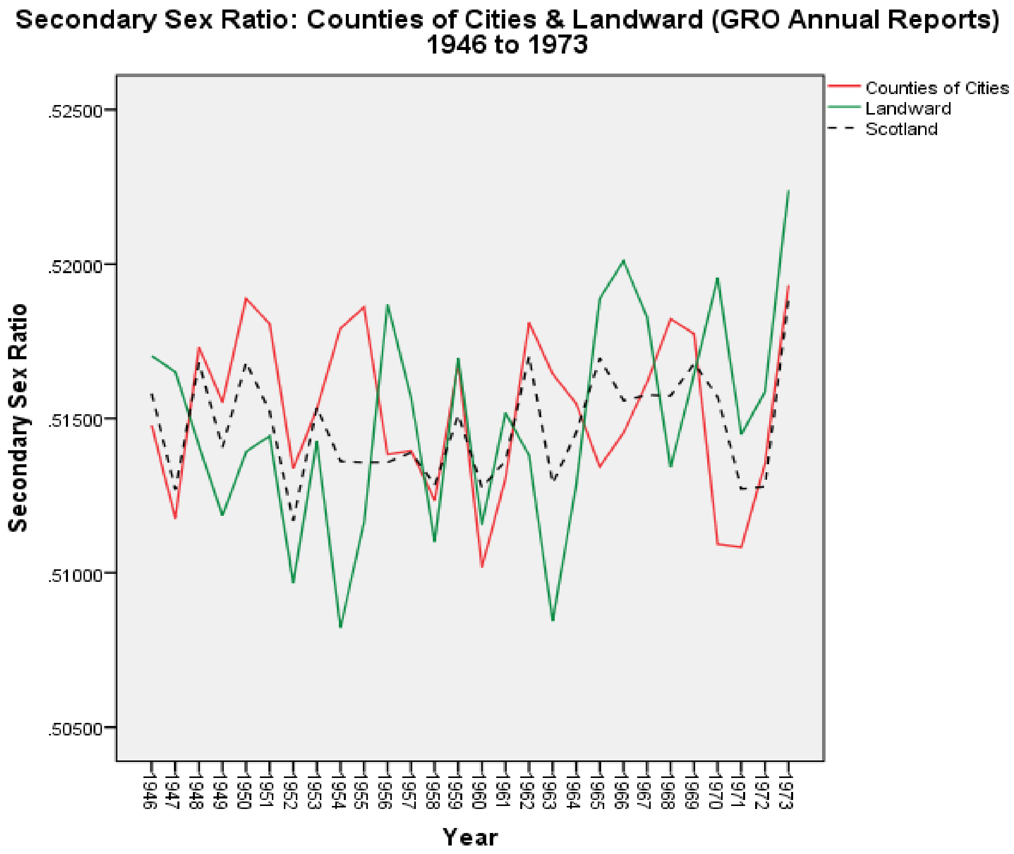
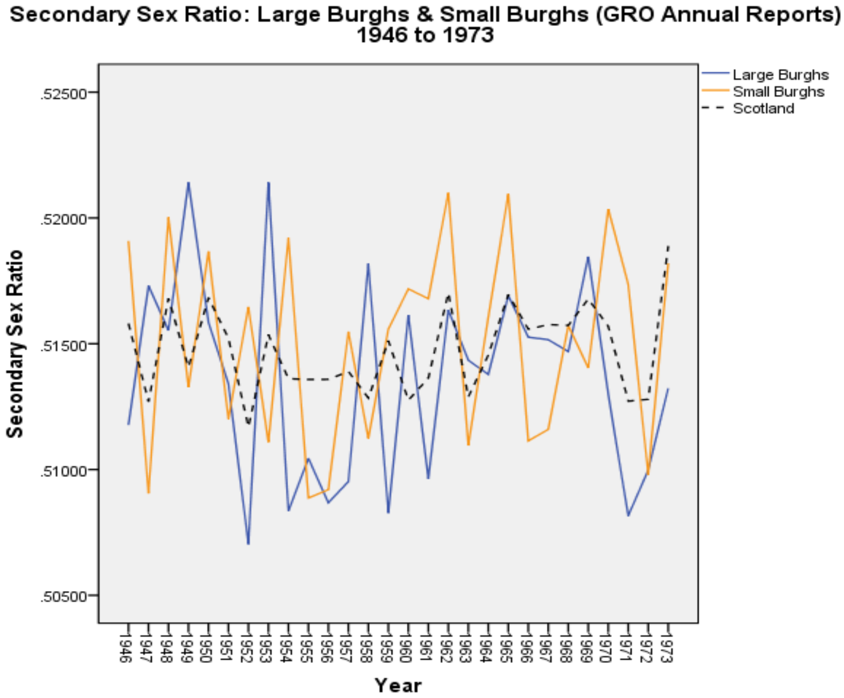


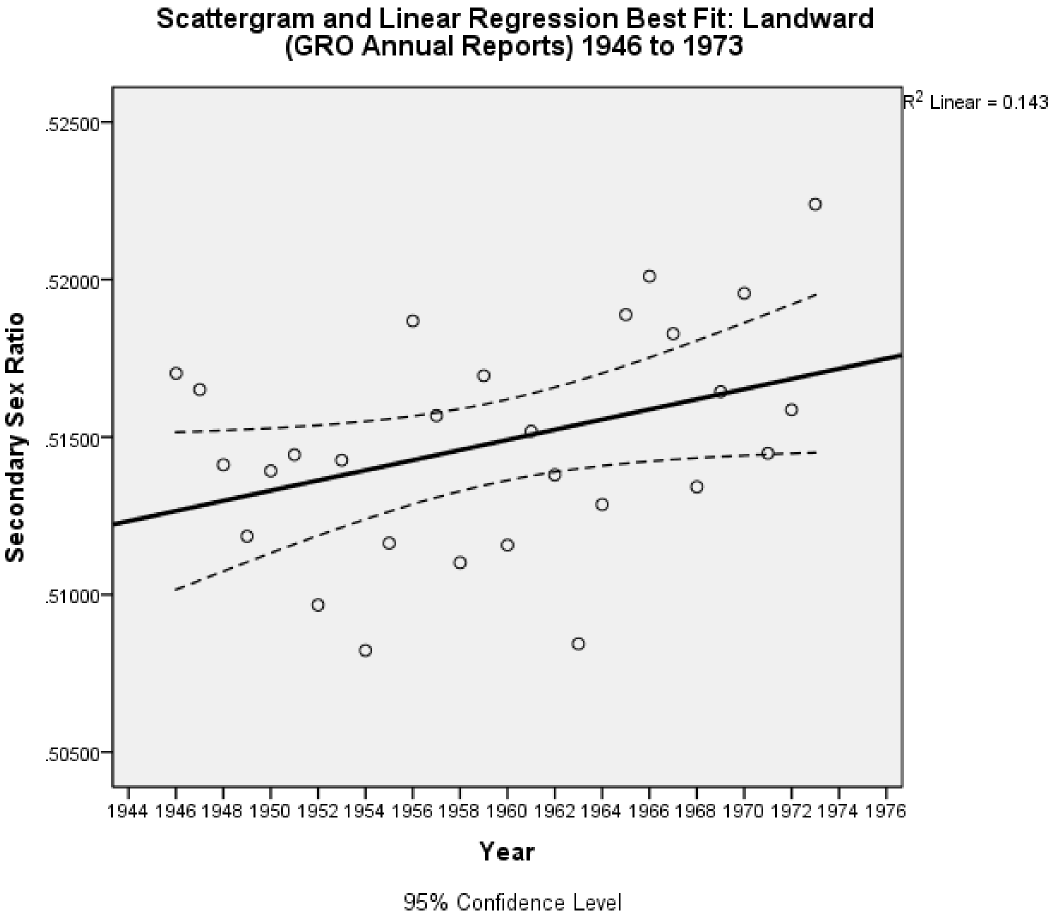
Figure 9: Secondary Sex Ratio: Large Burghs & Small Burghs 1946 to 1973



(*Data source*: General Register Office for Scotland, Annual Reports 1946 to 1973, Accessed at Ladywell House, Ladywell Road, Edinburgh)

In further investigation of the Landward secondary sex ratio, linear regression of the temporal trend is shown in Figure 10 together with corresponding models in Tables 16 & 17. A significant upward trend is observable in the graph and established in the model, with an R^2 coefficient of 0.143 in predicting such variation based on 95% confidence interval. This model is important in establishing an *increase* in sex ratio over each year from 1946 to 1973 for rural areas of Scotland, in contrast to the national ratio which was commencing a downward change.

Figure 10: Scattergram and Linear Regression Best Fit: Landward 1946 to 1973



Tables 17 & 18: Linear Regression Co-efficients: Secondary Sex Ratio & Time in Years: Landward Geography (GRO Annual Reports) 1946-1973

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.378[a]	.143	.110	.00330572

a. Predictors: (Constant), Year
b. Dependent Variable: Landward

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.512	.001		399.237	.000
	Ordinal Year	.000	.000	.378	2.084	.047

a. Dependent Variable: Landward

(*Data source*: General Register Office for Scotland, Annual Reports 1946 to 1973, Accessed at Ladywell House, Ladywell Road, Edinburgh)

5.2.4 Regional: Health Board Areas

Examination is also made of data available on annual secondary sex ratio for Health Regions in Scotland from 1973 to 2010. A distinct advantage of analysing these figures is their partial temporal alignment with the declining male birth proportion period discussed in 5.2. Cumulative figures for annual secondary sex ratio for Health Regions in Scotland from 1973 to 2010 (Table 19) show significant upward skewing of sex ratio for the Highland region and lower male birth proportion figures for the Borders and Forth Valley. Although the Forth Valley difference in male birth proportion is not significant (Z score 1.1522) it is potentially important given the region's total births constitute over 5% of the national figure.

Table 20 provides 'two proportions' testing on Health Region birth totals for each of the 5 years where the national ratio was lowest between 1973 and 2010. The 3 statistically lowest (by Z score) birth sex ratio regions for each year are shown in the Table. The Forth Valley Health Board area is identified in 4 of the 5 years with a significantly low sex ratio at the 95% confidence level in 2008. The larger Lothian region is also important with male birth proportion significantly low in 2010 (99% CI) and near significance in 2001. Both regions thus display a potential downward *levering* effect on the national sex ratio, with Forth Valley more consistently over the 1973 to 2010 period. As stated in SEPA's 2008 national air quality report 'the majority of Scotland's industrial oxides of nitrogen are generated in east central Scotland' and by far the largest emitters are located in the Forth Valley and Lothian regions (Scottish Environmental Protection Authority 2008). Scotland's only air quality management area for industrial pollution is declared at Grangemouth in the Forth Valley region, the location of a large manufacturing industrial complex and refinery. As such, the identification of these regional birth sex ratio trends tentatively suggests a possible environmental link to the national decline in male birth proportion.

Table 19: Large Sample Significance Testing of Birth Sex Ratio by Health Region 1973 To 2010

Health Board Region	*Male Births*	*Total Births*	*Birth Sex Ratio*	*Z Score**	*2 Tail significance test**
Ayrshire + Arran	83,308	162,732	0.51193373	0.4842	0.6282
Borders	21,015	41,372	0.50795224	1.8561	0.0634
Dumfries + Galloway	30,505	59,640	0.51148558	0.5159	0.6059
Fife	79,231	154,753	0.51198361	0.4354	0.6632
Forth Valley	62,268	121,888	0.51086243	1.1522	0.2492
Grampian	116,390	226,240	0.51445368	1.7244	0.0846
Greater Glasgow + Clyde	300,875	586,978	0.51258310	0.0383	0.9694
Highland (incl. A+B)	67,503	130,407	0.51763326	3.5705	†0.0004
Lanarkshire	136,182	265,541	0.51284736	0.2854	0.7754
Lothian	173,421	338,582	0.51219793	0.3883	0.6978
Orkney, Shetland & Western Isles	15,738	30,946	0.50856330	1.3953	0.1629
Tayside	85,087	165,627	0.51372663	0.9215	0.3568

* ‘2nd proportion or large sample: Yorkshire & Humber Male Births 1974 to 2010 / Yorkshire & Humber Total Births (1,185,114/2,312,167)

† Significant at 99% confidence interval

(*Data source*: Statistical Information Services, Demography Division, National Records of Scotland, via E-mail)

Table 20: Large Sample Significance Testing of Birth Sex Ratio by Health Region for 5 'downward' years of National Birth Sex Ratio

Year 1984

National/Health Authority	*Male Births*	*Female Births*	*Total Births*	*Birth Sex Ratio*	*Z score **	*2 Tail Significance Test **
Scotland	*33144*	*31962*	*65106*	*0.5091*	*2.5752*	***0.01*
Ayrshire + Arran	2401	2401	4802	*0.5000*	2.0001	†0.0455
Tayside	2411	2390	4801	*0.5022*	1.6987	0.0894
Highland	1932	1900	3832	*0.5042*	1.2745	0.2025
Year 1994						
Scotland	*31399*	*30257*	*61656*	*0.5093*	*2.4306*	*†0.0151*
Fife	2028	2082	4110	*0.4934*	2.6901	**0.0071
Forth Valley	1615	1594	3209	*0.5033*	1.2691	0.2044
Lanarkshire	3506	3409	6915	*0.5070*	1.2377	0.2158
Year 2001						
Scotland	*26786*	*25741*	*52527*	*0.5099*	*1.9705*	*†0.0488*
Lothian	4208	4123	8331	*0.5051*	1.7016	0.0888
Forth Valley	1414	1403	2817	*0.5020*	1.3291	0.1838
Borders	529	537	1066	*0.4962*	1.1911	0.2336
Year 2008						
Scotland	*30570*	*29471*	*60041*	*0.5092*	*2.4526*	*†0.0142*
Forth Valley	1711	1743	3454	*0.4954*	2.2413	†0.025
Borders	558	580	1138	*0.4903*	1.6291	0.1033
Grampian	3193	3130	6323	*0.5050*	1.5050	0.1323
Year 2010						
Scotland	*29872*	*28919*	*58791*	*0.5081*	*2.9042*	***0.0037*
Lothian	4921	4903	9824	*0.5009*	2.6639	**0.0077
Grampian	3158	3096	6254	*0.5050*	1.5008	0.1334
Forth Valley	1672	1658	3330	*0.5021*	1.4270	0.1536

*2nd proportion or large sample: Scotland 1974 to 1979 Male Births/ Scotland 1974 to 1979 Total Births (204745/397934)

†Significant at the 95% Confidence Interval

** Significant at the 99% Confidence Interval

(*Data source*: Statistical Information Services, Demography Division, National Records of Scotland, via E-mail)

5.2.5 Regional: Local Authority Areas

Birth sex ratios for all 32 Local Authority areas in Scotland can be calculated with extraction of data from the Scottish Neighbourhood Statistics web-portal. The cumulative mean birth sex ratios for all local authority areas from 1991 to 2010 are presented in Table 19 including the birth totals and the Scotland ratio/total. Local Authority areas are listed according to birth sex ratio values, from smallest to largest, with the first six being Scottish Borders, South Lanarkshire, Falkirk, East Lothian, Dumfries & Galloway, and Renfrewshire. Testing of differences in the regional and national proportions was also undertaken. These calculations then produced an alteration in the earlier order from Table 20 with the lowest sex ratio population returning significance being South Lanarkshire (Z Test, 2.327 and 2 Tail Sig., 0.0327). Scottish Borders, Falkirk, Renfrewshire, Dumfries & Galloway, and East Lothian, then follow with 2 tail significance values outside the 95% confidence interval.

Table 19 - Secondary Sex Ratios for Local Authority Areas (All) 1991 to 2010

Local Authority	Male Births 1991-2010	Total Births 1991-2010	Birth Sex Ratio
Scottish Borders	11292	22309	0.5062
South Lanarkshire	35324	69525	0.5081
East Lothian	10670	20986	0.5084
Falkirk	17537	34492	0.5084
Dumfries & Galloway	15370	30219	0.5086
Renfrewshire	20291	39881	0.5088
Midlothian	9605	18834	0.5100
South Ayrshire	11090	21727	0.5104
East Renfrewshire	9880	19354	0.5105
Fife	40281	78893	0.5106
Stirling	9122	17849	0.5111
West Lothian	21233	41542	0.5111
East Ayrshire	13986	27361	0.5112
Clackmannanshire	5890	11522	0.5112
Angus	12003	23463	0.5116
Glasgow City	75120	146792	0.5117
Eilean Siar	2703	5281	0.5118
North Ayrshire	15752	30769	0.5119
Scotland	**592967**	**1158056**	**0.5120**
Edinburgh City	51514	100452	0.5128
Argyll & Bute	8987	17523	0.5129
Orkney Islands	2071	4038	0.5129
Aberdeen City	24215	47184	0.5132
North Lanarkshire	41228	80214	0.5140
Aberdeenshire	26978	52466	0.5142
Perth & Kinross	14301	27803	0.5144
West Dunbartonshire	11374	22090	0.5149
Dundee City	17281	33562	0.5149
Moray	10200	19762	0.5161
Highland	24208	46849	0.5167
East Dunbartonshire	10937	21156	0.5170
Shetland Islands	2793	5390	0.5182
Inverclyde	9731	18768	0.5185

(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk)

Table 20: Lowest Secondary Sex Ratios for Local Authority Areas 1991 to 2010: Z Test and Significance

Local Authority	Male Births 1991-2010	Total Births 1991-2010	Birth Sex Ratio	Z Test	2 Tail Sig.
South Lanarkshire	35324	69525	0.5081	2.137	0.0327
Scottish Borders	11292	22309	0.5062	1.799	0.0720
Falkirk	17537	34492	0.5084	1.393	0.1636
Renfrewshire	20291	39881	0.5088	1.356	0.1750
Dumfries & Galloway	15370	30219	0.5086	1.243	0.2135
East Lothian	10670	20986	0.5084	1.039	0.2743

(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk)

5.2.6 Sub-Regional: Parliamentary Constituencies

In Table 21, 6 of the 79 Scottish parliamentary constituency areas are displayed with the lowest cumulative birth sex ratios over the 1999 to 2007 period. Two proportion significance testing is also performed with the Yorkshire & Humber equivalent birth totals included as the 'comparator' proportion. Results from Table 21 indicate downward skewing of sex ratio for 5 constituencies, all of which are within the earlier identified local authority areas of Scottish Borders, Renfrewshire, South Lanarkshire and Falkirk. The Roxburgh & Berwickshire area displays a very low proportion of male births (Z Score 3.111, 2 Tail sig. 0.0019) and differs from the other identified constituencies in being distinctly rural. All other areas outside the 95% confidence interval of prediction highlighted, Paisley North, Hamilton North & Bellshill, Falkirk West and Glasgow Rutherglen, are peripheral urban areas in Scotland's Central Belt, a region strongly identified with the legacy of pollution from heavy manufacturing industry.

Table 21: Secondary Sex Ratios for Constituencies (Scottish Parliament) 1999 to 2007

Parliamentary Constituency	Male Births 1999-2007	Total Births 1999-2007	Birth Sex Ratio	Z Test	2 Tail Sig.
Roxburgh & Berwickshire	2224	4552	0.4889	3.111	**0.0019**
Paisley North	3100	6243	0.4966	2.386	**0.0172**
Hamilton North & Bellshill	3538	7088	0.4993	2.103	**0.0355**
Falkirk West	3719	7433	0.5003	1.950	0.0511
Aberdeen South	3482	6942	0.5016	1.679	0.0931
Glasgow Rutherglen	3409	6785	0.5024	1.522	0.1281

(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk)

5.3 Hypothesis Testing of Area-based Indicators

In the second stage of the analysis bi-variate correlation testing and stratified analyses are performed of Scottish Neighbourhood Statistics and Scottish Index of Multiple Deprivation retrospective area-based indicators (see 3.5.2). This permits the testing of the null hypotheses which were stated in 4.2.1. Birth figures were extracted from SNS to produce 6505 sex ratio calculations for all datazones in Scotland (administrative units of 600 – 1000 persons, see 3.4.1), based on male and total births from 2002 to 2009. This dependent variable could then be analysed with the corresponding indicators measured for datazones in Scotland, thus forming the independent variables.

5.3.1 Data Conversion

Conversion of data to a more compatible numerical format for investigating statistical relationships in quantitative research is frequently necessary. Figure 11 is a histogram of the frequency of the 6505 secondary sex ratio values showing it’s relatively *normal distribution* and thus compatibility for testing correlations with independent variables.

However, the male birth proportion calculations for every datazone do not account for the proportional significance of each sex ratio based on total births for each zone. For example, a pre-dominantly rural zone may only record up to 50 births for the 2002 to 2009 period whereas a datazone in an urban growth area may display 4 times such number. Such differences could be addressed by performing significance testing on all 6505 'samples' or datazones, however this could add statistical complication to the simple models required. This problem also only arises with correlation testing as stratified analysis permits sex ratio calculation for larger group categories, or collection of datazone totals, rather than individual datazones. An alternative dependent variable was therefore created as a 'check and balance' to results from the sex ratios of all 6505 'small-areas'. This consisted of 'low weighting out' (LWO) sex ratio dependent variable whereby only datazones where total births were greater than 50 for the 2002 to 2009 period were included. This excludes to a limited extent sex ratio values which are artificially skewed as they are formulated from low number of birth cases. Figure 12 illustrates the relatively normal distribution of this conversion with a total 4258 datazones included. A consequence of using the 'low weighting out' option is the likely bias of urban rather than rural areas given the propensity for lower birth totals in smaller population areas outside of cities and towns. Both dependent variables are therefore included in bi-variate correlations.

Figure 11: Datazone secondary Sex Ratios: Frequency and Normal Distribution

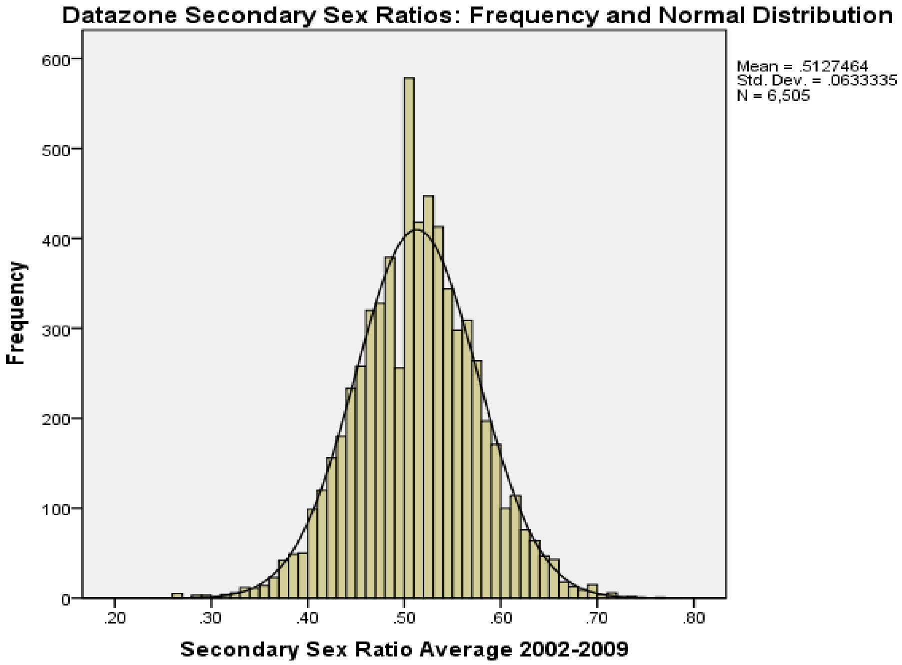
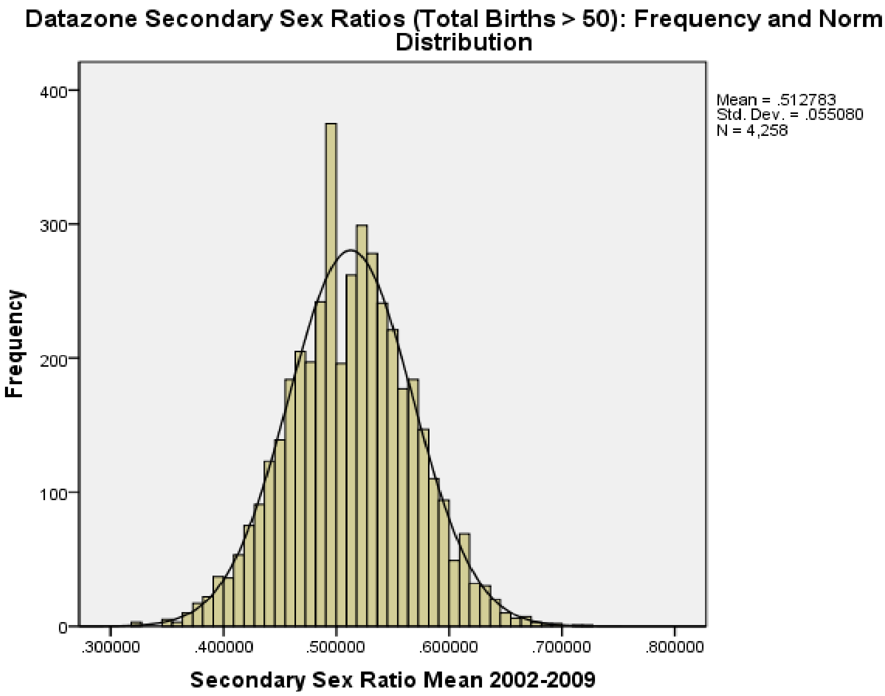


Figure 12: Datazone Secondary Sex Ratios (Total Births >50): Frequency and Normal Distribution



The Scottish Government presents the multiple deprivation index as a rank, either for every datazone, 1 (most deprived) to 6505 (least deprived), or by vigintile, separating the ranks into 20 ordinal categories, 1 (most deprived) to 20 (least deprived). This was used to undertake the Spearmans Rank Correlation testing.

5.3.2 Multiple Deprivation (Socio-Economic Status): Hypothesis 2

Ordinal ranking of SIMD is conducive to Spearman's Rank correlation testing which is displayed in Table 22. The absence of any correlation between male birth proportion and deprivation is demonstrated with both these co-efficients showing values of -0.004 (sig, 2-tailed 0.803) and -0.005 (sig. 2-tailed 0.663).

Table 22: Spearman's Rank Correlation – Secondary Sex Ratio and Index of Multiple Deprivation Rank (2006)

Correlations

			Secondary Sex Ratio Average 2002-2009	SSRLowWeightingOut	Index of Multiple Deprivation Rank : 2006
Spearman's rho	Secondary Sex Ratio Average 2002-2009	Correlation Coefficient	1.000	1.000**	-.005
		Sig. (2-tailed)	.	.	.663
		N	6505	4258	6505
	SSRLowWeightingOut	Correlation Coefficient	1.000**	1.000	-.004
		Sig. (2-tailed)	.	.	.803
		N	4258	4258	4258
	Index of Multiple Deprivation Rank : 2006	Correlation Coefficient	-.005	-.004	1.000
		Sig. (2-tailed)	.663	.803	.
		N	6505	4258	6505

**. Correlation is significant at the 0.01 level (2-tailed).

The final deprivation figures (Table 23) show the stratified analysis of male birth proportion and SIMD vigintiles. As demonstrated with lack of bi-variate correlation there is no gradated change in sex ratio from lower to higher deprivation categories.

There is, however, a significantly elevated male/female birth ratio of 0.5235 for the highest vigintile, the least deprived and wealthiest sub-group of the Scottish population. The Z proportion test on this ratio, with over 20,000 births, reveals a less than 1% probability of such skewing occurring by chance (Z=3.2079, 2 Tail sig. =0.0013).

Upwards skewing, however, also occurs for the 2nd *most* deprived group with a birth sex ratio of 0.5185 which is significant at the 95% confidence interval. Further, significantly reduced male birth proportions (95% CI) are calculated for vigintiles 13 and 17. A ‘book-ends’ effect is therefore possibly discernible with low male birth proportion attributed to the middle categories, and thus middle class population, with increased sex ratios observed at both extremities of social class. A line graph illustration of this effect is displayed in Figure 15.

Table 23: Large Sample Significance Testing of Secondary Sex Ratio 2001 to 2009 by Datazone SIMD Vigintile

Vigintile	*Deprivation*	*Male Births*	*Total Births*	*Birth Sex Ratio*	*Z Score†*	*2 Tail Significance Test†*
1	*Most Deprived*	17529	34272	0.5115	0.3297	0.7417
2		16736	32278	0.5185	2.1355	*0.0327
3		15081	29401	0.5129	0.1862	0.8523
4		13636	26695	0.5108	0.5035	0.6146
5		13208	25865	0.5107	0.5449	0.5858
6		13087	25535	0.5125	0.0399	0.9682
7		12685	24983	0.5077	1.4345	0.1514
8		12245	23787	0.5148	0.7228	0.4698
9		11880	23133	0.5136	0.3480	0.7278
10		12130	23593	0.5141	0.5269	0.5982
11		11943	23379	0.5108	0.4618	0.6442
12		11245	21856	0.5145	0.6148	0.5387
13		10830	21422	0.5056	1.9614	*0.0498
14		12004	23361	0.5138	0.4383	0.6612
15		12490	24180	0.5165	1.2663	0.2054
16		11963	23529	0.5084	1.1863	0.2355
17		11138	22041	0.5053	2.0537	*0.04
18		12416	24285	0.5113	0.3424	0.732
19		11211	22129	0.5066	1.6816	0.0926
20	*Least Deprived*	11206	21404	0.5235	3.2078	**0.0013

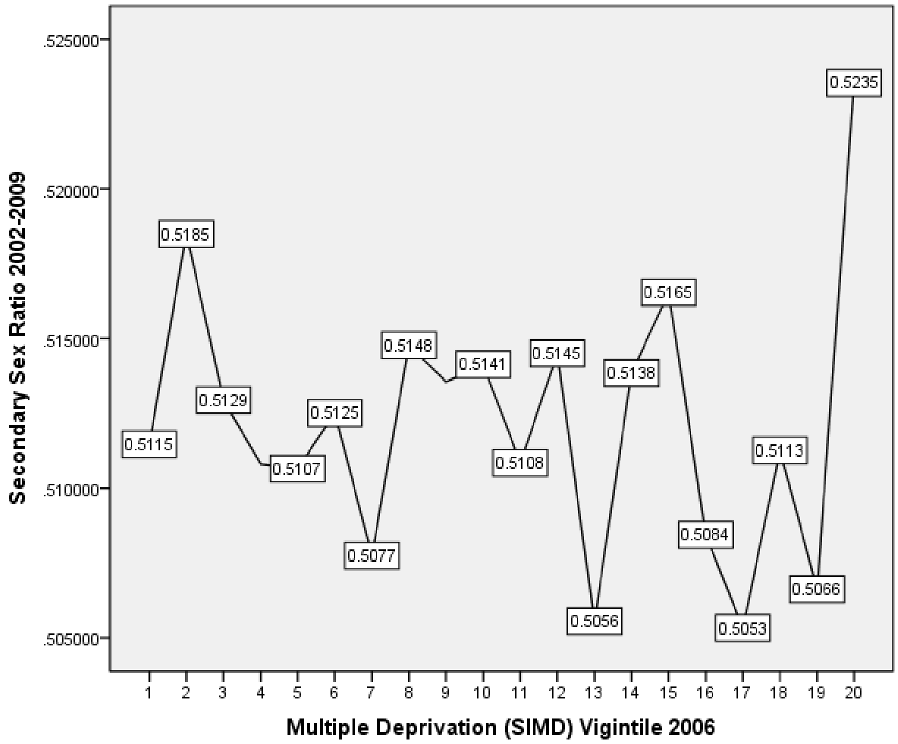
†2nd proportion or large sample: Yorkshire & Humber 2001 to 2009 Male Births/Total Births (281708/549798)

*****Significant at 95% Confidence interval

**Significant at 99% Confidence interval

(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk & Scottish Index of Multiple Deprivation, Scottish Government. www.scotland.gov.uk/topics/statistics/SIMD)

Figure 13: Line Graph of Secondary Sex Ratio 2001 to 2009 by Datazone SIMD Vigintile



(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk & Scottish Index of Multiple Deprivation, Scottish Government. www.scotland.gov.uk/topics/statistics/SIMD)

5.3.4 Maternal Age – Hypothesis 3

As shown in Table 24 there is no correlation between percentage of first-time mothers aged 35 and over in datazones with both measurements of secondary sex ratio. In Table 25 a stratified analysis of male birth proportion is also displayed for the percentage of first time mothers aged 35 in datazones. Six categories are assigned based on intervals of 5% up to 20%, followed by 20% to 30% and 30% to 60%. Falsification of the maternal age null hypothesis would be sustained by significantly low sex ratios for the high percentage categories which do not occur. The graph shows no significant differences over the groups of percentages thus supporting the null results from the correlation coefficient testing.

Table 24: Pearson Correlation – Secondary Sex Ratio and Percentage of First Time Mothers aged 35 and over by Datazone

Correlations

		Secondary Sex Ratio Average 2002-2009	SSRLowWeightingOut	PercentOver35Mothers
Secondary Sex Ratio Average 2002-2009	Pearson Correlation	1	1.000**	-.015
	Sig. (2-tailed)		.000	.231
	N	6505	4258	6505
SSRLowWeightingOut	Pearson Correlation	1.000**	1	.022
	Sig. (2-tailed)	.000		.151
	N	4258	4258	4258
PercentOver35Mothers	Pearson Correlation	-.015	.022	1
	Sig. (2-tailed)	.231	.151	
	N	6505	4258	6505

**. Correlation is significant at the 0.01 level (2-tailed).

Table 25: Large Sample Significance Testing of Birth Sex Ratio 2001 to 2009 by Percentage Mothers aged 35 in Datazone

Maternal Age Categories	*Percentage Mothers aged 35 and above*	*Male Births*	*Total Births*	*Birth Sex Ratio*	*Z score†*	*2 Tail significance test†*
1	0 to 5%	68234	132897	0.5134	0.6877	0.4917
2	5.01`% to 10%	61791	120518	0.5127	0.2058	0.8369
3	10.01% to 15%	47362	92656	0.5112	0.6901	0.4902
4	15.01% to 20%	32991	64693	0.5100	1.1657	0.2437
5	20.01% to 30%	30565	59615	0.5127	0.1494	0.8812
6	30.01% to 60%	13720	26749	0.5129	0.1699	0.8651

†2nd proportion (large sample): Yorkshire & Humber 2001 to 2009 Male Births/Total Births (281708/549798)

(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk & Scottish Index of Multiple Deprivation, Scottish Government. www.scotland.gov.uk/topics/statistics/SIMD)

5.3.5 Population Stress –Hypothesis 4

Stress as a potential determinant for alteration in sex ratio is tested through the compilation of 3 indicators to constitute *neighbourhood proxy stress* scores/levels: prescribed drugs for depression and anxiety; SIMD crime rate; and drug misuse hospital admissions (see 3.5). An important characteristic of this proxy measure of local chronic stress is its auto-correlation with multiple deprivation, as demonstrated in Table 26. A Spearman's rank test of correlation shows a very high significant association (co-efficient - 0.769, sig.- 0.00) between neighbourhood stress levels and the SIMD index. Results from testing of neighbourhood stress variable are therefore similar to the multiple deprivation values and trend. A Pearson correlation test reveals no association between proxy neighbourhood stress values and both datazone secondary sex ratio measures. Further examination by stratification of the stress variable in Table 27 shows no significant variation in birth sex ratio. For a moderate level of neighbourhood stress there is a slight movement of local sex ratio downwards. As for the deprivation trend there is a slightly discernible 'book-end' effect of increased sex ratios at the highest and lowest ends of the proxy neighbourhood stress composite variable.

Table 26: Spearman's Rank and Pearson's Correlation – Neighbourhood Stress and SIMD Index (2006)

Correlations

			Neighbourhood_Stress	Index of Multiple Deprivation Rank : 2006
Spearman's rho	Neighbourhood_Stress	Correlation Coefficient	1.000	-.769**
		Sig. (2-tailed)	.	.000
		N	6505	6505
	Index of Multiple Deprivation Rank : 2006	Correlation Coefficient	-.769**	1.000
		Sig. (2-tailed)	.000	.
		N	6505	6505

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

		Secondary Sex Ratio Average 2002-2009	SSR Low Weighting Out	Neighbourhood Stress
Secondary Sex Ratio Average 2002-2009	Pearson Correlation	1	1.000**	.015
	Sig. (2-tailed)		.000	.238
	N	6505	4258	6505
SSRLowWeightingOut	Pearson Correlation	1.000**	1	.002
	Sig. (2-tailed)	.000		.903
	N	4258	4258	4258
Neighbourhood_Stress	Pearson Correlation	.015	.002	1
	Sig. (2-tailed)	.238	.903	
	N	6505	4258	6505

**. Correlation is significant at the 0.01 level (2-tailed).

Table 27: Large Sample Significance Testing of Birth Sex Ratio 2001 to 2009 by Datazone Proxy Neighbourhood Stress

Stress Categories	*Stress Levels*	*Male Births*	*Total Births*	*Birth Sex Ratio*	*Z score†*	*2 Tail significance test†*
1	0 to 137	44651	87318	0.5114	0.5622	0.574
2	138 to 285	46661	91401	0.5105	1.0183	0.3085
3	286 to 505	49303	96519	0.5108	0.8384	0.4018
4	506 to 912	52740	102576	0.5142	0.9931	0.3207
5	913 to 5605	60837	118393	0.5139	0.8724	0.383

†2nd proportion (large sample): Yorkshire & Humber 2001 to 2009 Male Births/Total Births (281708/549798)

(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk & Scottish Index of Multiple Deprivation, Scottish Government. www.scotland.gov.uk/topics/statistics/SIMD)

5.3.6 Maternal Smoking: Hypothesis 5

The variable of maternal smoking is measured by the ‘percentage of women smokers at first ante-natal appointment’. The mean percentage was calculated from 2001 to 2009, a year before the cumulative sex ratio time period (2002-2009) because recording of smoking status is possible in the year preceding birth occurrence. As previously discussed, levels of maternal smoking are positively related to deprivation incidence and in fact ‘actual’ smoking consumption is often under reported. Table 28 illustrates the auto-correlation of maternal smoking and derivation with a very high Pearsons co-efficient of 0.744 at the 99% confidence interval. Also important in this table is the correlation of maternal smoking with ‘number of low weight births’, indicating a statistical relationship in maternal health and peri-natal outcome variables for area-based measurement. For secondary sex ratio, however, this is not replicated with Table 29 demonstrating no correlation between the variables and therefore confirmation of the null hypothesis. This is further validated with large sample significance testing and stratified analysis showing no large differences in birth sex ratio for levels of maternal smoking in ‘small areas’. The possible exception to this is the lower male birth proportion for category 3, once again demonstrating a middle concentration in socio-economic area-based indicators of moderate decreases in sex ratio.

Table 28: Pearson Correlation – Percentage of Female Smokers at First Ante-Natal Clinic, No. of Low Weight Births and Multiple Deprivation

Correlations

		Maternal Smoking (%)	No. of Low Weight Births	Log Deprivation Score
Maternal Smoking (%)	Pearson Correlation	1	.358**	.744**
	Sig. (2-tailed)		.000	.000
	N	6505	6505	6484
No. of Low Weight Births	Pearson Correlation	.358**	1	.448**
	Sig. (2-tailed)	.000		.000
	N	6505	6505	6484
LogDeprivationScore	Pearson Correlation	.744**	.448**	1
	Sig. (2-tailed)	.000	.000	
	N	6484	6484	6484

**. Correlation is significant at the 0.01 level (2-tailed).

Table 29: Spearman’s Rank - Percentage of Female Smokers at First Ante-Natal Clinic and Secondary Sex Ratio by Datazone

Correlations

			Maternal Smoking (%)	Secondary Sex Ratio Average 2002-2009	SSR Low Weighting Out
Spearman's rho	Maternal smoking (%)	Correlation Coefficient	1.000	-.005	-.001
		Sig. (2-tailed)	.	.664	.936
		N	6505	6505	4258
	Secondary Sex Ratio Average 2002-2009	Correlation Coefficient	-.005	1.000	1.000**
		Sig. (2-tailed)	.664	.	.
		N	6505	6505	4258
	SSR Low Weighting Out	Correlation Coefficient	-.001	1.000**	1.000
		Sig. (2-tailed)	.936	.	.
		N	4258	4258	4258

**. Correlation is significant at the 0.01 level (2-tailed).

Table 30: Large Sample Significance Testing of Birth Sex Ratio 2001 to 2009 by Percentage of Women Smoking at first ante-natal clinic by Datazone

Maternal Smoking Categories	*% Women Smoking at 1st ante-natal booking*	*Male Births*	*Total Births*	*Birth Sex Ratio*	*Z score†*	*2 Tail significance test†*
1	0 - 10%	58300	113835	0.5121	0.14731	0.8829
2	10.01 – 20%	66816	130031	0.5138	0.94861	.34282
3	20.01 – 30%	52809	103515	0.5102	1.31474	.18860
4	30.01 – 40%	41386	80978	0.5111	0.69492	.48710
5	< 40.01%	35352	68769	0.5141	0.83308	.40480

†2nd proportion (large sample): Yorkshire & Humber 2001 to 2009 Male Births/Total Births (281708/549798)

(*Data Source:* Scottish Neighbourhood Statistics, Scottish Government. www.sns.gov.uk & Scottish Index of Multiple Deprivation, Scottish Government. www.scotland.gov.uk/topics/statistics/SIMD)

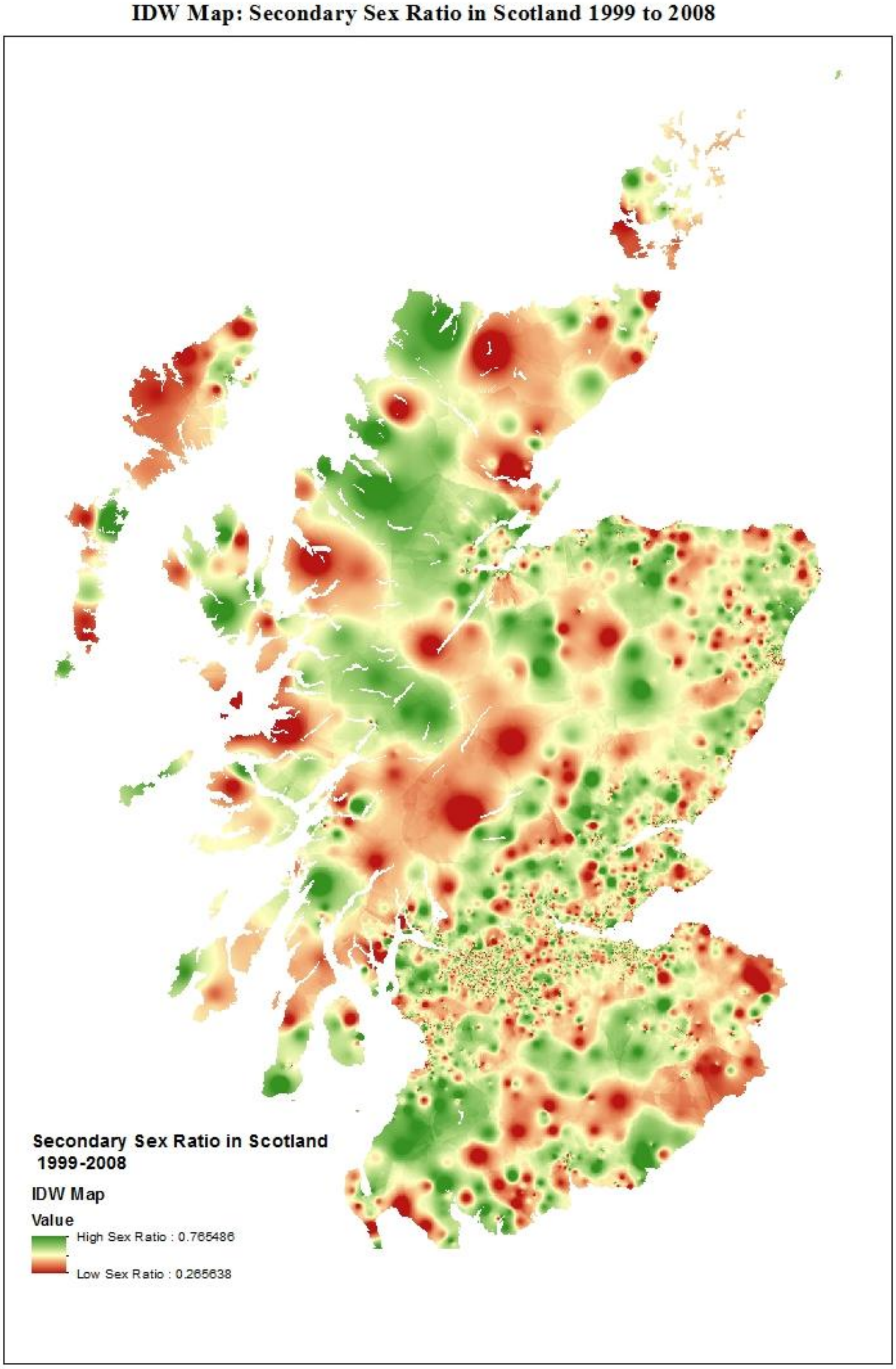
5.4 Spatial Analysis

The third section of the Results chapter includes mapping of birth sex ratio geography and closer examination of potential influencing factors, principally endocrine disruptor pollution and exposure. A central region case study is chosen as the suitable geography and accompanying data for further analysis of male birth proportion trends. Maps were produced with GIS software ArcMap 10 and ancillary tables constructed in Excel and SPSS. All co-ordinate and polygon data were accessed from the EDINA data library at the University of Edinburgh (www.edina.ac.uk).

5.4.1 Scotland: Inverse Distance Weighting Map

Map 1 utilises the inverse distance weighting technique (IDW) to display the mean birth sex ratio distribution across Scotland for 1999 to 2008. The population centroids for 6505 datazones with corresponding secondary sex ratios for 1999 to 2008 are combined to construct this map (see 4.6.4). IDW is an interpolation method which determines cell values on the basis of linear weighting, the weight being a function of inverse distance. For Figure 16, birth sex ratios are plotted at the centroid points which are further away from a raster cell exact less value on that cell and vice versa for closer ratio points. No limit was set on interpolation (default setting) and therefore cells further away from the centroid points have 'exaggerated' values which ostensibly denote remote and rural areas. Notwithstanding this rural and remote area bias, the advantage of IDW in substantially addressing the 'mappable unit area' problem delivers important demarcations of low birth sex ratio, particularly in rural regions. Some areas are more than likely artificially skewed given their remoteness, such as far north Scotland (Sutherland) and southern Highlands (Trossachs). However, the concentration of red pixilation for South East Scotland, from Berwickshire through the southern Borders onto Dumfries and Galloway, is an area of greater population settlement and corresponds with the earlier birth sex ratio trends for local authorities and parliamentary constituencies. Of particular interest is that this raster plotting covers a large part of the Solway Tweed river catchment, a key area of environmental concern for Scotland with regard to water pollution. This illustrates the benefits of IDW mapping in transgressing "fixed" geographical areas, such as datazones, and signifying trends across an entire geographical surface (e.g. Scotland).

Map 1: Inverse Distance Weighting Map – Secondary Sex Ratio (1999 to 2008) by Datazone Population Centroid



5.4.2 *Endocrine Disruptor Pollution: Hypothesis 1*

Maps 2, 3 and 4 display the final modelling of endocrine disruptor air pollution for SEPA registered sites with low datazone sex ratios at 3 levels of significance; 0.05, 0.10 and 0.25 respectively. The detailed methods of how the polygon model overlays for air pollution were produced are stated in 4.7, including relevant appendices. The polygons are gradated by 5 categories of cumulative discharge, measured in kg, for 2002 and 2004 to 2010. As expected the Grangemouth industrial complex contains 3 of the 5 highest SPRI emitters for the Central Scotland region, including the BP oil refinery, INEOS manufacturing and Dalkia utilities. High air emissions of endocrine disruptors are not exclusive to Grangemouth, however, with the Norbord plastics factory in Stirling and waste recycling in Airdrie also displaying large discharge totals. It is important to note that the bulk of these declarations concern nitrous oxide and carbon monoxide and that the toxicity of the pollutants and extent of their endocrine disrupting properties are not quantitatively measured. A key consideration therefore is the identification and thus importance of every site in releasing endocrine disrupting agents. An example of this is emitters of dioxins, which is a highly toxic group of chemicals to humans though total releases are small.

Observance for spatial concentration of low birth sex ratios and convergence with the endocrine disruptor models is the approach undertaken for testing of the pollution hypothesis. A discernible pattern for low male birth proportion distribution in the maps is its confinement to the central and urban areas with the possible exception of rural regions to the south of Hamilton, near Strathaven and Stonehouse. This corresponds with the results of parliamentary constituency and local authority sex ratios described earlier i.e. South Lanarkshire , Falkirk West and Hamilton North & Belshill (Table22). The location of SPRI registered emitters and industries in, or adjacent to, urban areas, such as Grangemouth, Stirling, Falkirk and Hamilton, illustrates a rudimentary regional spatial association with endocrine disruptor air pollution and low birth sex ratio. There is, however, no consistent, definitive overlapping of the air pollution models with datazones that have low birth sex ratios at all significance levels.

The maps also demonstrate important localised areas of sex ratio skewing which include the following:

(i) a large datazone on the east of Rutherglen and Cambuslang which has significantly skewed birth sex figures in a population growth area, as shown in Table 31 below.

Table 31 Rutherglen South Datazone S1006036 Birth Sex Ratio

Datazone	S01006036
Intermediate Geography	Halfway, Hallside and Drumsagard
Parliamentary Constituency	Rutherglen South
Total Births	418
Total Male Births	188
Total Female Births	230
Secondary Sex Ratio	0.44976
Z Test	2.5621
2 Tailed Significance	0.0104

(ii) Concentration of low male birth proportion figures for neighbourhoods in the Falkirk local authority area, relatively close to the Grangemouth industrial complex and also at Bonnybridge (West of Falkirk). The significant Bonnybridge skewing replicates results from an earlier study with 1980s data and is centred around the location of a former waste incinerator plant which was investigated by Scottish authorities because of local health concerns (Williams, Lawson et al. 1992); and

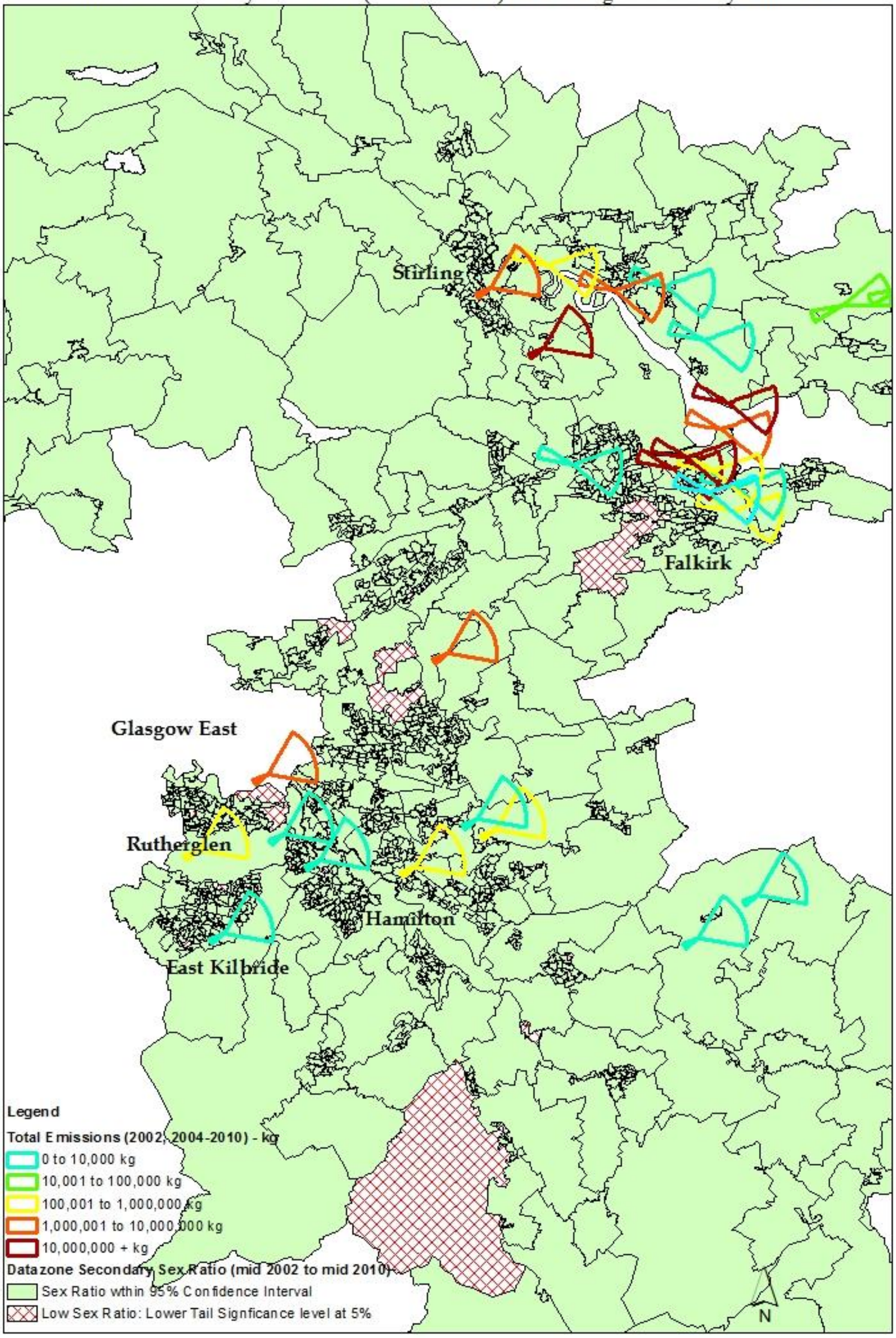
(iii) a large section of adjoining datazones with low birth sex ratios within the 75% confidence interval ($p < 0.25$) in the central belt zone between Airdrie and Falkirk. The co-located Greengairs Landfill site is a large emitter of nitrogen dioxide and carbon monoxide.

These possible sentinel changes in local reproductive health, indicated by significant downward sex ratio skewing, are also in regional areas affected by land contamination. The legacy of large scale disposal of waste chemicals and metals during heavy industrialisation in the central belt of Scotland is a potential source of exposure possibly through soil, water and air, particularly with remediation works for brownfield land development.

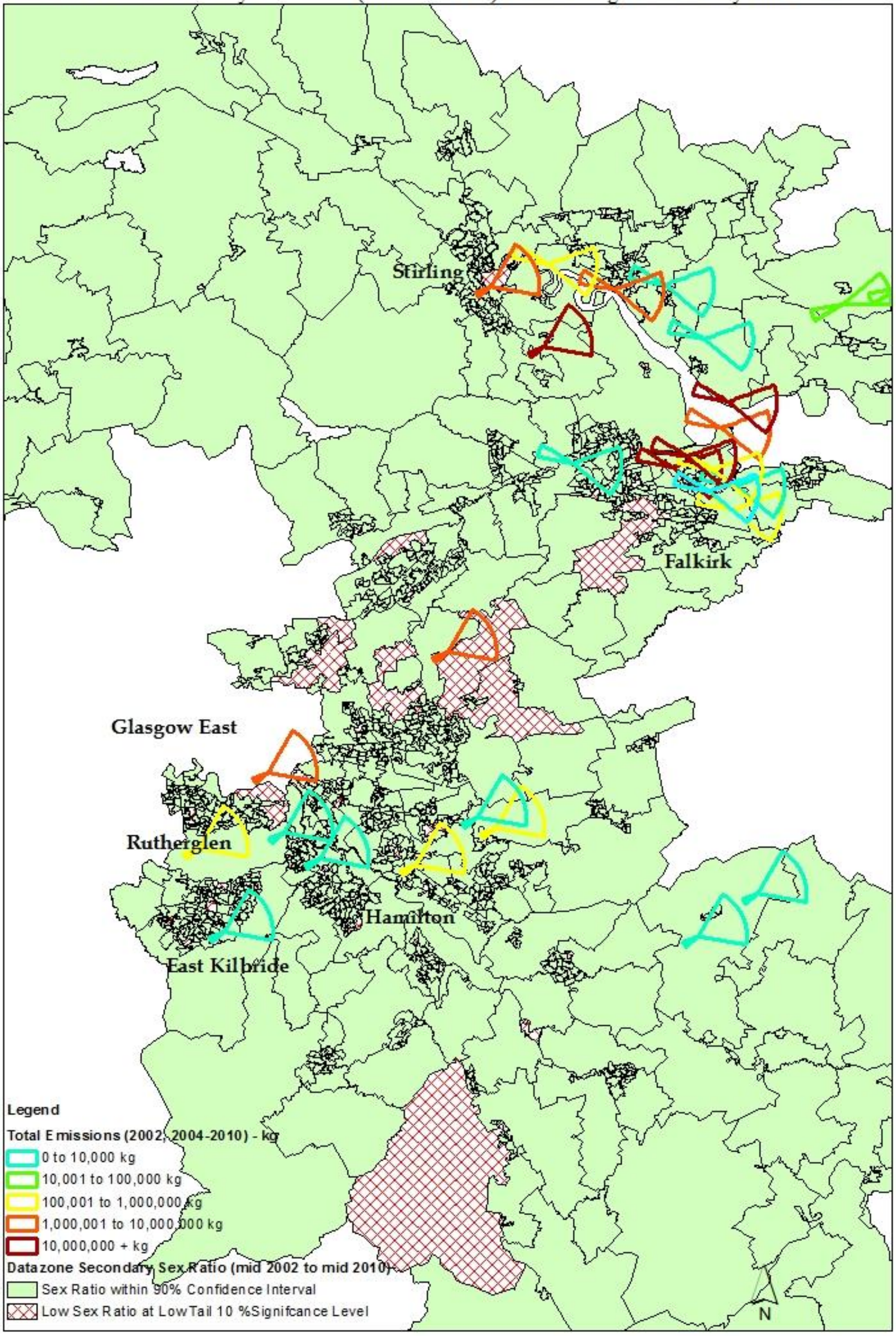
5.4.3 Multi-Factorial – Endocrine Disruptor Pollution and Multiple Deprivation: Hypothesis 5

By overlaying maps of datazone multiple deprivation vigintiles, low secondary sex ratio (p value <.10) and air pollution models, an examination of the multi-factorial hypothesis can be made. Given the association of deprivation with maternal smoking and neighbourhood deprivation the SIMD vigintiles provide concordant representation for all these variables. The local maps for Grangemouth, Falkirk and Stirling (Map 5) and Rutherglen, Hamilton and Cumbernauld (Map 6) though, reveal very few spatial associations.

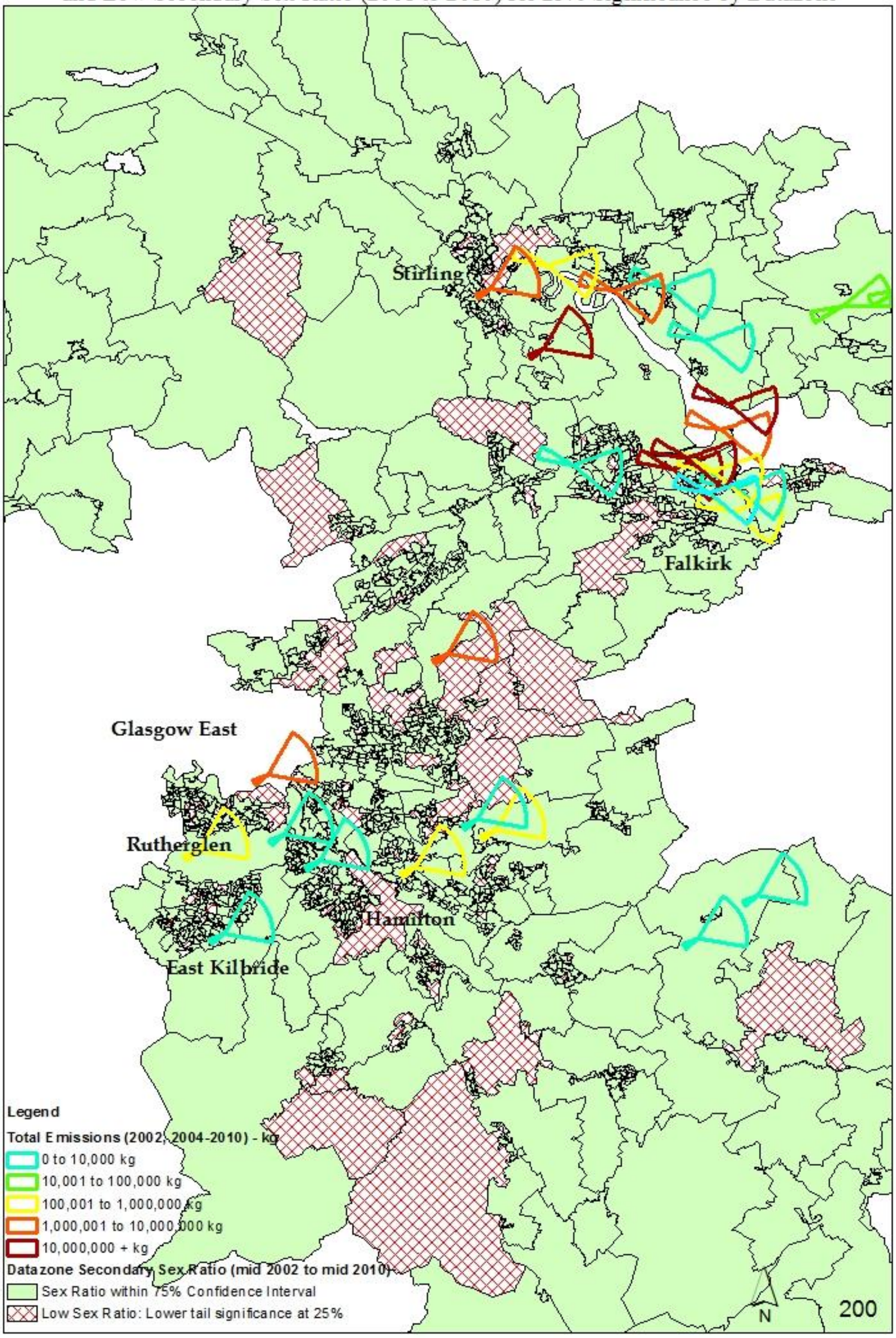
Map 2: Central Scotland - Cumulative Endocrine Disruptor Pollution (2002, 2004-2010) and Low Secondary Sex Ratio (2001 to 2010) for 5% Significance by Datazone



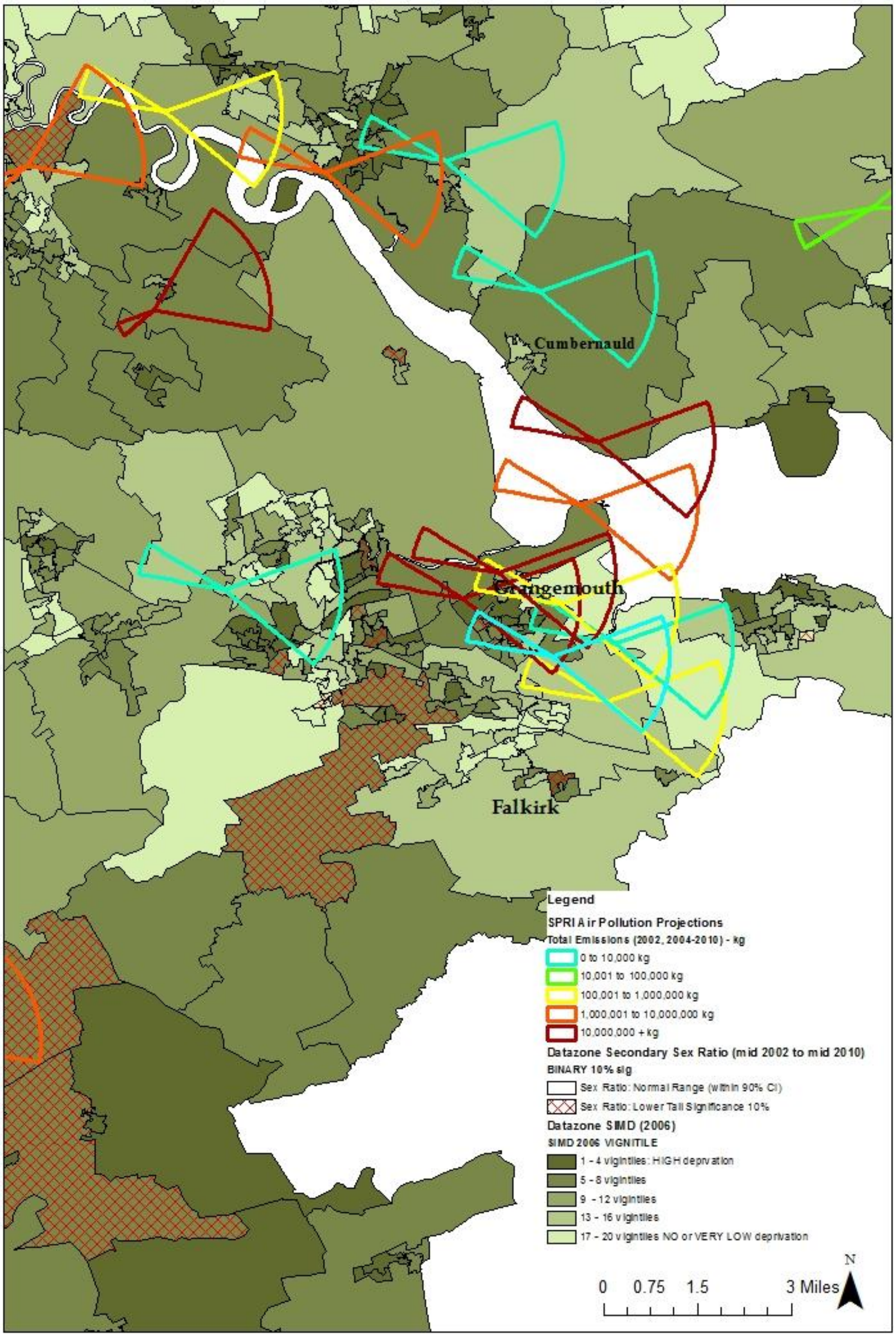
Map 3: Central Scotland - Cumulative Endocrine Disruptor Pollution (2002, 2004-2010) and Low Secondary Sex Ratio (2001 to 2010) for 10% Significance by Datazone



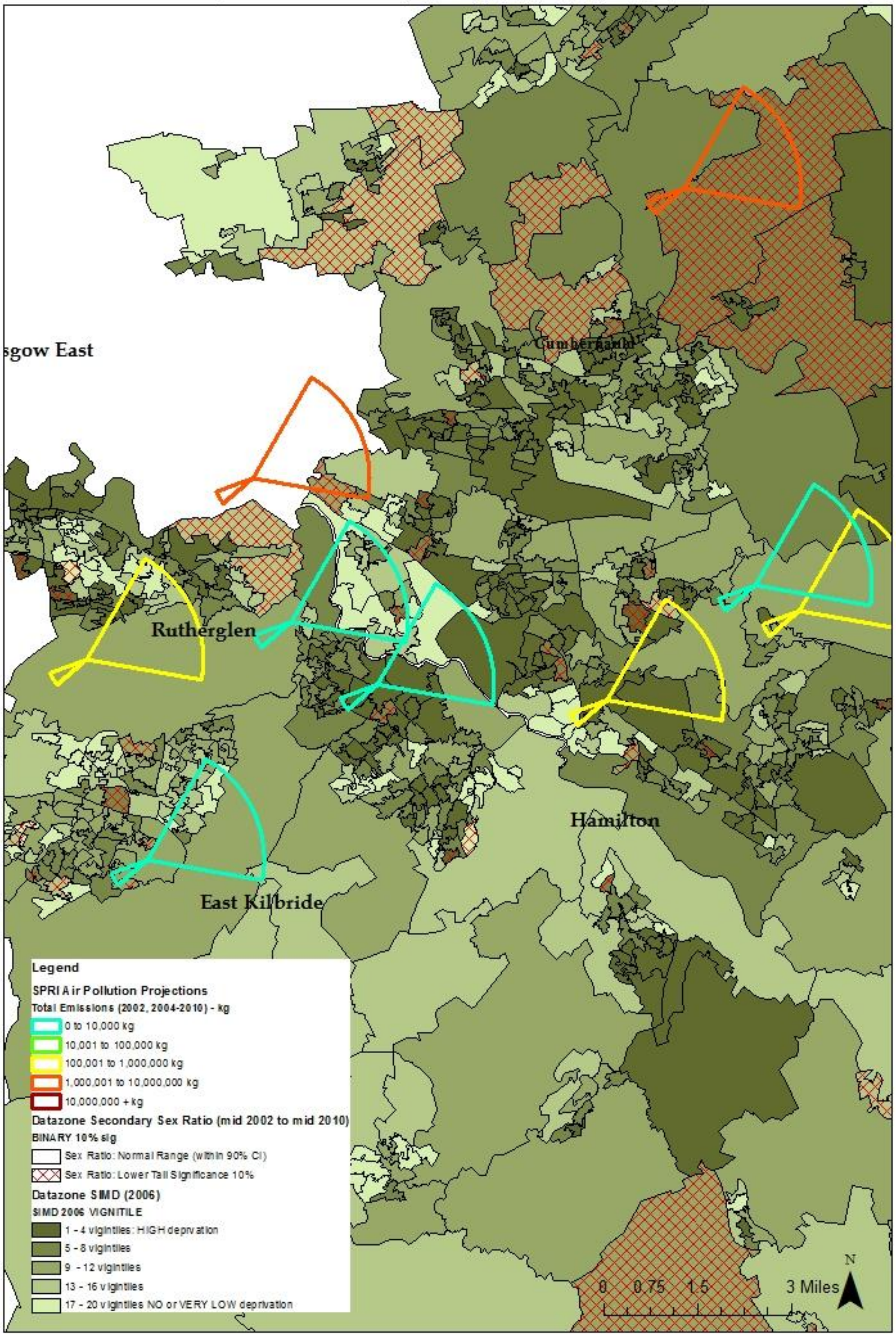
Map 4: Central Scotland - Cumulative Endocrine Disruptor Pollution (2002, 2004-2010) and Low Secondary Sex Ratio (2001 to 2010) for 25% Significance by Datazone



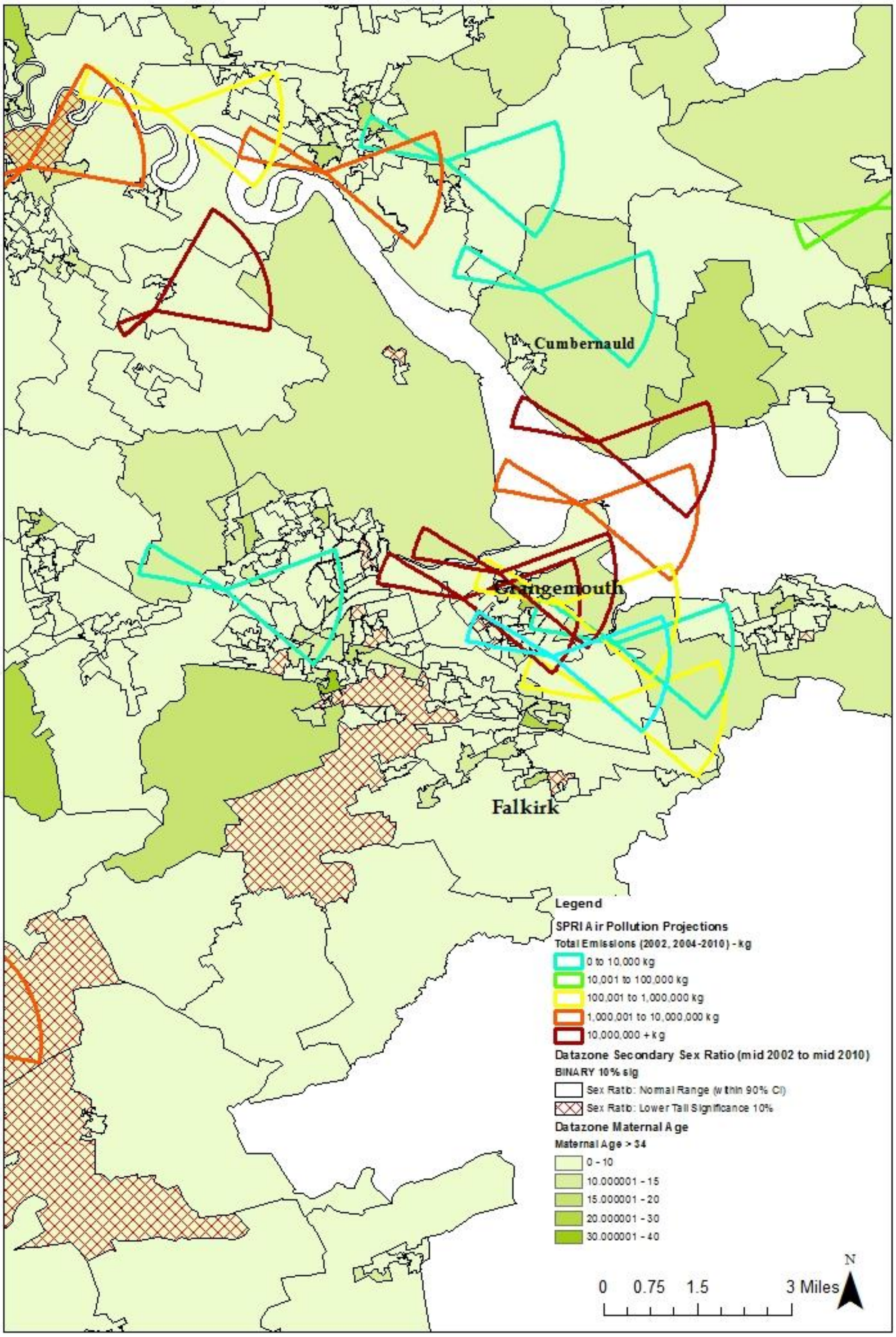
Map 5: Grangemouth, Falkirk - Cumulative Endocrine Disruptor Air Pollution (2002, 2003 - 2010), SIMD (2006) Vigintiles and Low Birth Sex Ratios (mid 2002 - mid 2011) 10% significance by Datazone



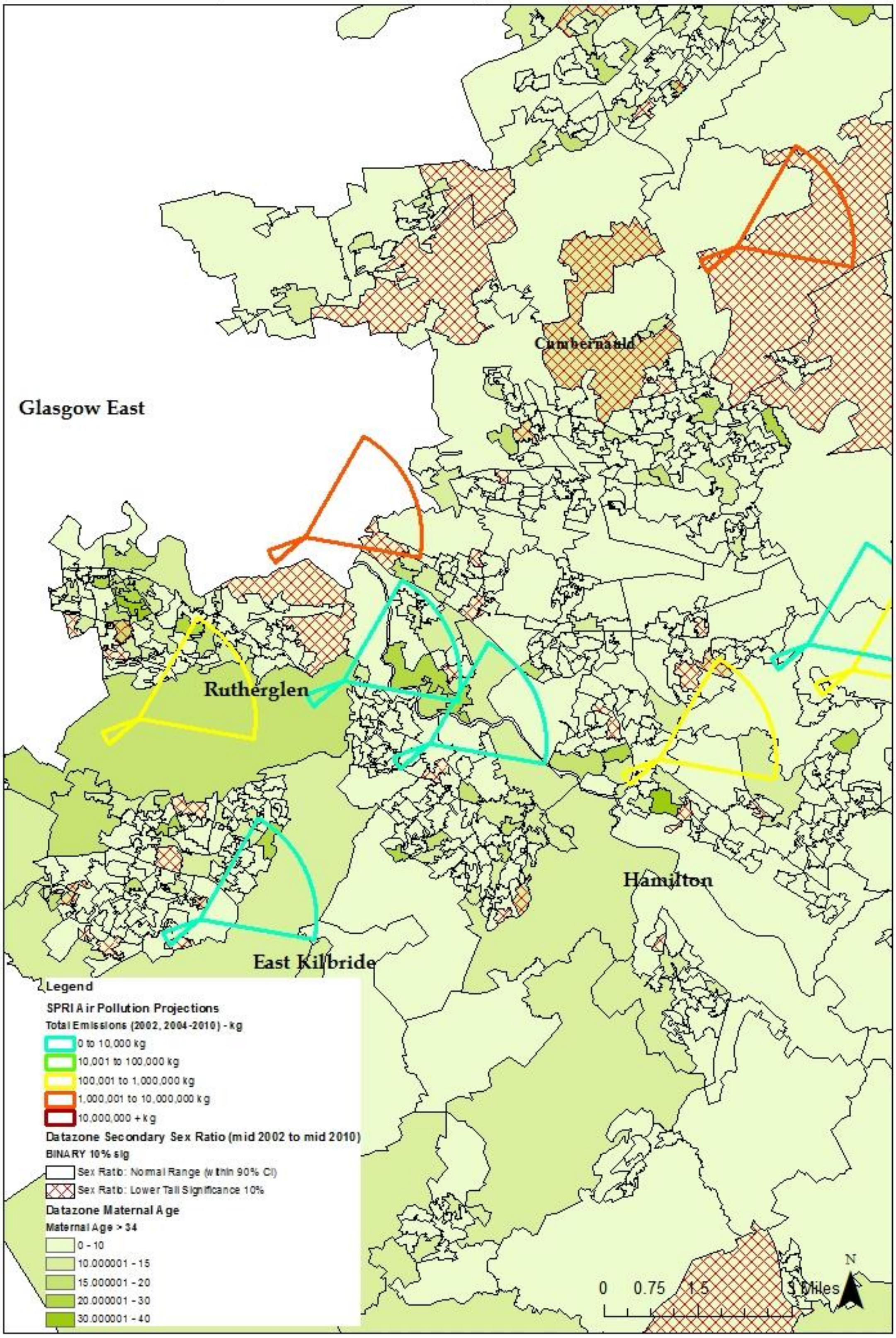
Map 6: Rutherglen, Hamilton - Cumulative Endocrine Disruptor Air Pollution (2002, 2003 - 2010), SIMD (2006) Vigintiles and Low Birth Sex Ratios (mid 2002 - mid 2011) 10% significance by Datazone



Map 7: Grangemouth, Falkirk - Cumulative Endocrine Disruptor Air Pollution (2002, 2003 - 2010), Percentage of Mothers Aged >34 years and Low Birth Sex Ratios (mid 2002 - mid 2011) 10% significance by Datazone



Map 8: Rutherglen, Hamilton - Cumulative Endocrine Disruptor Air Pollution (2002, 2003 - 2010), Percentage of Mothers Aged >34 years and Low Birth Sex Ratios (mid 2002 - mid 2011) 10% significance by Datazone



5.4.4 Multi-Factorial – Endocrine Disruptor Pollution & Maternal Age: Hypothesis 5

Maps 7 and 8 also constructed by including categories for the percentage of first-time mothers aged 35 and over in datazones. As stated earlier (4.6.1), this offers a proxy indication of the percentage of older parents and thus can be included as an interactive variable with potential endocrine disruptor exposure. As per the SIMD local maps, however, there is no area convergence of both these variables with significantly low birth sex ratios, suggesting interactive effects as unlikely.

5.5 Summary of Results

The investigation outlined above, of secondary data on birth sex ratio in Scotland and possible factors influencing its alteration is *exploratory* in scope. A comprehensive analysis of secondary sex ratio trends and geography, together with statistical examination of potential determinants and a geo-analytical review of endocrine disruptor pollution and male birth proportion have yielded significant results for discussion. The following findings are relevant to a continuing scientific debate on the declining proportion of male births phenomenon, the potential association with the environmental endocrine hypothesis and the possible implication of demographic/socio-cultural or health-related factors:

- The historical trend in birth sex ratio for Scotland is similar to other industrialised countries in experiencing a sustained increase in the 20th century up to the 1960s and then a significant decline to the present time;
- A moderate increase in birth sex ratio occurred for distinctly non-metropolitan areas in Scotland from 1946 to 1973;
- A significant regional 'levering' of the national secondary sex ratio downwards occurred during years of low male birth proportion for the 1973 to 2010 period, primarily though not exclusively linked to the Forth Valley and Lothian Health Regions;
- A statistically significant mean birth sex ratio in the South Lanarkshire local authority area is evident for the years 1991 to 2010;
- A common trend is observable of downwards sex ratio skewing for geographic units within the Scottish Borders, Falkirk, South Lanarkshire and Renfrewshire regions i.e. parliamentary constituencies, datazones, and local authority areas;

- A very low male birth proportion is evident in the Roxburgh and Berwickshire Scottish parliamentary constituency area between 1999 and 2007;
- No association exists between secondary sex ratio in 6505 datazones and the equivalent area based indicators of multiple deprivation, advanced maternal age, maternal smoking and neighbourhood status;
- A stratified analysis of area based measurement of multiple deprivation indicates a significantly elevated birth sex ratio for the second least and most deprived population groups presenting a potential 'book-ends' effect for socio-economic status on sex ratio;
- Spatial analysis shows regional concentration of low skewed sex ratio datazones within urban areas in the central region of Scotland with some zones overlapping or proximate to models of point source cumulative endocrine disruptor pollution; and
- Localised incidences of low skewed sex ratios are observed on peripheral areas of Rutherglen and Falkirk and also in close proximity to the Greengairs Landfill waste facility near Airdrie.

These key results are discussed in following chapter in relation to each of the null hypotheses generated from Chapter 4. Comment is made on this summary together with findings from previous research in order to address the pivotal research questions for this study.

Chapter 6: Discussion

6.1 Introduction – Results Summary

Secondary data and spatial-temporal analysis of endocrine disruptor air pollution, multiple deprivation, proxy population stress and maternal age do not reveal statistically significant associations in this study with lower proportions of male births in small areas. However, projections of source emissions of cumulative endocrine disruptor air pollution in Central Scotland overlap, or are near to, low birth sex ratios in datazones that are significant or close to significance. Further, regional variance of male birth proportion over the last 40 years in Scotland illustrates levering of the national sex ratio downwards amongst some regional health board areas, in particular Forth Valley which exhibits the greatest concentrations of industrial pollutant emissions in Scotland. For the deprivation analysis, a significant upward skewing in birth sex ratio for the least deprived and most prosperous cohort is derived, though no consistent pattern across the stratification is shown with the second most deprived group also displaying high male birth proportion.

6.2 Research Hypotheses

This chapter will examine each of the hypotheses raised at the beginning of the thesis with regard to the study results as well as the scientific debates and literature surrounding each factor/hypothesis. The hypotheses of population stress and advanced maternal age are firstly considered, followed by discussion on smoking and multiple deprivation, and then finally a fundamental schema for this research; evaluation of the environmental endocrine hypotheses. The multi-factorial hypothesis is discussed amongst these subjects, particularly in view to pollution, with perspectives from relevant biological, social and environmental science.

The chapter also examines the wider context of the research, not only the viability of the male birth proportion statistic as a reproductive marker in populations but also scientific debates concerning reproductive health and fertility in North American and European countries. A premise for undertaking the research, and linked to the first hypothesis stated above, is Davis's proposition of linking endocrine disruption to male birth proportion decline in industrialised countries (Davis, Gottlieb et al. 1998). Together with observance of localised sex ratio decline in communities vulnerable to environmental hazard, this proposed linkage has received significant attention in North America, including academic commentary on male reproductive health and the environmental endocrine hypothesis (Mocarelli, Gerthoux et al. 2000; Safe 2000; Davis 2002; Mackenzie, Lockridge et al. 2005; Daniels 2006; CBC News 2008). Further epidemiological trends and health events have also been linked to endocrine disruption including infant neuro-behavioural problems (Grandjean and Landrigan 2006; de Cock, Maas et al. 2012), small size for gestational age and low weight births (Hertz-Picciotto, Charles et al. 2005; Kezios, Liu et al. 2012; Tsukimori, Uchi et al. 2012), postnatal growth (Lamb, Taylor et al. 2006), increased incidence of breast and prostate cancer (Sasco 2001; Soto and Sonnenschein 2010), and earlier onset of pubertal development (Schoeters, Den Hond et al. 2008; Den Hond, Dhooge et al. 2011). More commonly, however, in the scientific literature, discussion is confined to male reproduction, specifically rising testicular cancer incidence, cryptochordism and hypospadias, and declining sperm quality, including a potential tri-partite continuum of these health phenomena called testicular dysgenesis syndrome (TDS) (Krimsky 2000; Skakkebaek, Rajpert-De Meyts et al. 2001). There is also concentration, particularly in andrology commentary and research on adverse male fertility as symptomatic of the ubiquity of hormone disrupting compounds (Andersson, Jorgensen et al. 2008; Joensen, Skakkebaek et al. 2009). The critical question therefore is whether extension of the environmental endocrine hypothesis, to include declining sex ratios in selected industrialised countries alongside sentinel male reproductive health deterioration, is supported by the current evidence. This necessitates the examination and testing of the other hypotheses which have been proposed (listed above), as well as those exclusively focused on the determination of the ‘normal’ male excess for secondary sex ratio such as the evolutionary biological theory of Trivers-Willard.

A concluding focus should also be the extent to which male birth proportion decreases in population are principally a *male* problem, encompassing such characteristics as TDS and poor fertility, or are predominantly linked to female reproductive health, or a combined parental aetiology. In addition, a recent hypothesis on environmentally mediated genetic changes in sperm also requires consideration. In order to achieve a *holistic* assessment of reproductive health, deliberation on each of socio-economic, health-related and demographic hypotheses and potential factors is required before examining the environmental pollution evidence and arguments. Importantly, not only can such hypotheses be rejected or validated as singly explaining secondary sex ratio in populations but also suggestive as *contributory* to multi-factorial effects and outcomes.

6.3 Population Stress

The scoping review revealed that population stress, examined predominantly through time series analyses, associates strongly with antecedent low sex ratio periods. A proxy measurement of chronic neighbourhood stress was constructed incorporating measures on prescriptions for anxiety, local crime rate and alcohol abuse. With the exception of a significantly reduced male birth proportion for group 4 of the neighbourhood stress categories, a moderate stress rank, the results are unremarkable. There was no correlation between these variables, either by Spearmans rank or Pearsons co-efficient, for the 6,505 datazones in Scotland. Similarly there was little evidence of a combined pollution and stress effect from the spatial analysis conducted of the 5 local authority areas in Central Scotland.

It is feasible a ‘book-end’ pattern eventuates with neighbourhood stress and male birth proportion for the population groups, whereby the lowest sex ratios are observed in the middle level for measurement of local stressors and related treatments. Interestingly, this trend is also slightly discernible for the deprivation vigintiles, an auto-correlated variable, with raised birth sex ratios at the upper and lower ends of multiple deprivation, the wealthiest and poorest groups in the Scottish population. The difficulty in sustaining the ‘book-end’ proposition, however, is that there is no distinct gradated observance and sex ratio figures oscillate to either low, normal and high between neighbouring categories. Notwithstanding this, there is a significant downward deviation from the national sex ratio for persons living in areas exposed to moderate proxy neighbourhood stress from crime and drug misuse. Such a result though is interpreted with some degree of caution.

Impetus for inclusion of a local environment stress-related variable in the analysis is from theoretical literature and research on neighbourhood disorder, psychological stress and adverse birth outcomes (Hill, Ross et al. 2005; Farley, Mason et al. 2006). It is also concordant with scholarship on the 'developmental origins of health and disease' and 'life course approach' in epidemiology whereby epigenetic mechanisms in the intrauterine environment can influence health outcomes in later life (Gluckman, Hanson et al. 2007). Previous sex ratio research, however, is largely focused on *acute* events, both at the population level, such as natural disasters, or by individual or family circumstances, such as a partner's or close relative's illness or death. Stress is most often understood or framed as a temporal phenomenon, induced by particular event and then resolved over time. Inquiry conducted in male proportion, particularly time-series analyses undertaken by Catalano et al, ascribes to conceptualising stress on these terms. However, stressor longevity effects are more likely to contribute to apposite enduring sex ratio trends.

Obel, Henriksen et al. (2007) are exceptional in incorporating chronic stress as a determinant, though not explicitly stated, in an association between General Health Questionnaire scores and decreasing percentage of boys born. The findings for this retrospective cohort investigation, of 'moderate and common' as well as severe, levels of psychological stress potentially decreasing sex ratio is tentatively reinforced by this analysis of area-based data. Yet a problem in concluding that stress during pregnancy amongst female populations is likely to be involved in the widespread decline in the male proportion of male births is presuming that it has significantly increased for expectant women in societies over the post-war and recent period. Intuitively it would be reasonable to expect anxiety to magnify amongst pregnant women during and immediately after World War II but sex ratios rose in affected countries during this time (Graffelman and Hoekstra 2000). The 1960s rise in gender politics and modern challenges of workplace responsibilities for women during the reproductive phase of their lives is likely assumed in this proposition. Such a rationale for population stress elevation is likely sourced from media commentaries rather than sustained by robust epidemiological or sociological research.

Meanwhile a limitation for this study's use of a chronic environmentally-determined measure of population stress is its auto-correlation with other potential explanatory variables, namely multiple deprivation and maternal smoking incidence. This though does not preclude acute incidents of stress which may be experienced in neighbourhoods and that are not necessarily related to other socio-economic parameters. Any auto-correlation, however, could be partially resolved by applying multiple regression techniques (e.g. step-wise method) to unpack mutlicollinearity though lack of bi-variate correlations shown for the sex ratio dependent variable make such analysis impossible to commence. As discussed in Chapter 4 (4.8) this reflects the methodological difficulty in working with a fluctuating variable such as the secondary sex ratio, particularly measured according to geographical area. As an example, step-wise multiple regression with the SNS data-set can predict the reproductive indicator of *Low Weight Births*, by smoking, multiple deprivation, maternal age and nitrous dioxide pollution. Such indicative models cannot be constructed for secondary sex ratio hence a reliance on stratification in order to elucidate determining influences.

Environmental hazard also complicates attributing low sex ratio to stress exposure in some of the empirical studies on birth sex ratio. Earthquakes as population stress-inducing events are investigated in 3 studies though no consideration is given of consequent environmental hazards (Fukuda, Fukuda et al. 1998; Saadat 2008; Torche and Kleinhaus 2012). In the case of earthquake disasters severe pollution results from the debris of stored industrial and housing construction materials, sewerage disturbance, gas leaks, and a lack of availability of clean water (United States EPA 2012). In certain cases industrial plants can be destabilised and emit dangerous levels of pollutants, such as radioactivity at the Fukishima nuclear plant in Japan and sulphuric and phosphoric acid in Sichuan, China.

Similarly, pollution events and population stress can be concurrent and toxicology studies on sex ratio do not account for stress in exposure measurement. It is perfectly feasible during the industrial disaster at Seveso the nearby population was stress-affected as well as exposed to harmful levels of dioxins. Equally, accounting for compounding effects on reproductive outcomes and birth sex ratio of pollution and stress is valid. The maps constructed in Chapter 5, (Maps 5 and 6), with deprivation included as a proxy indicator stress indicator, indicate no manifestation of combined effects in Central Scotland. Theoretically, it is also possible to compare disasters which do not result in release of endocrine disrupting compounds with those where likely toxicity levels are high, thus ‘teasing out’ potential stress impacts.

Occupational health research may also be partly compromised in not disassociating both variables. Ruckstuhl, Colijn et al. (2010) categorise job groups by arbitrary levels of stress but neglect confounding from likely exposure to hazardous chemicals. In fact the resultant job categories with lowest male birth proportion, *Farming, fishing and natural resources* and *Trades, transport, construction*, have a greater propensity for occupational chemical hazard than other job categories, such as *Sales and services, Management*. For this study, environmental endocrine exposures and neighbourhood stress are both projected in Maps 5 and 6 through the auto-correlated variable of deprivation, for the Central Scotland spatial analysis. The maps display very little concentration of pollution and population stress with adjusted male birth proportion thus potentially repudiating any finding of combined effects. This analysis though only concerns a surrogate *chronic* measurement of stress defined by neighbourhood crime and drug misuse rather than stress-inducing. Acute pollution or natural disaster events present methodological challenges both in terms of timing of exposure measurement i.e. exactly at the time of the event, and how to quantify the stress levels. To a limited extent the September 11 studies may resolve some of these issues through investigation of a 'man-made' disaster which has no extensive follow-up pollution in the subject area of the entire city of New York, and also measuring sex ratio effects remotely in California on the basis of a 'communal bereavement' hypothesis (Catalano, Bruckner et al. 2005; Catalano, Bruckner et al. 2006; Bruckner, Catalano et al. 2010).

The precise impact of stress on reproductive health, pregnancy and secondary sex ratio is not authoritative. A way to improving scientific understanding would be to establish *when* stress effects occur in the stages of reproduction, ostensibly spermatogenesis, conception, fertilisation, embryogenesis, 1st trimester pregnancy, 2nd and 3rd trimester pregnancy, and birth. The sex ratio research varies on this basis, according to the biological mechanisms hypothesised and observations returned. Lack of sperm motility is proposed as instrumental to a drop in male births following an acute Balkan conflict; drops in coital rate amongst populations are suggested in earlier studies on earthquakes; whilst variable times of sex ratio downward alteration, either 1st, 3rd or 5th months of pregnancy, are demonstrated for proposed preferential spontaneous abortion of male foetuses. The most prominent hypothesis in the research literature is the latter, of early pregnancy, which is partially confirmed by a highly cited case-control study concluding that stressful events are strongly linked to the adverse peri-natal outcome of miscarriage (Maconochie, Doyle et al. 2007).

For the September 11 research there is difficulty in drawing inferences from results because there are no significant downward sex ratio changes other than at 4 months post the terrorist attack. The increased male fetal death rate for September 11 in New York confirms a ‘preferential’ *male* loss, however identification of the early second trimester phase as only instrumental to secondary sex ratio adjustment is not consistent with wider research evidence. There are also not plausible reproductive biological mechanisms developed to explain a universal spike in male fetal deaths at mid pregnancy and consequent sex ratio adjustment. The overwhelming evidence from the earlier review of potential mechanisms in reproductive and peri-natal health (Chapter 3) is that spontaneous pregnancy loss is most likely at the early stages of pregnancy. The problem for investigating causes of these occurrences is that miscarriage is not recorded by health authorities before 20 weeks of gravidity or for those outside hospitals. Maconochie, Doyle et al. (2007) therefore offer a formative approach to elucidating on adverse outcomes in peri-natal and reproductive health through innovative recruitment by extensive postal enquiry of the UK population.

The timing of conception and coital rate thesis receives far less equivocation in recent commentary with contradictory studies emerging since the original medical research (Grant 1998). On earlier stages of reproduction, there is limited scientific inquiry on decreased motility of spermatozoa or impaired spermatogenesis amongst males. One study, investigating only a relatively small cross-sectional group, establishes an association with cumulative workplace stress and male infertility (Sheiner, Sheiner et al. 2003). Holistic assessments of occupational stress and male reproductive health, combined with other determinants, such as endocrine disrupting chemicals and age, are potentially valuable future research programmes. A potentially very effective tool for such research projects is the new matrix for assessing occupational exposures, http://www.occideas.org/, though only if stress could also be added as an agent to the application.

The stratified and spatial analysis of neighbourhood stress in this thesis does not demonstrate a substantive link to decreases in sex ratio in small areas. One tentative hypothesis derived from the 'middle decline' in sex ratio, understood through a structural sociological perspective of modern Scotland, is of a 'pressurised' middle class experiencing stress through both demanding professional or para-professional work requirements, often for both partners, and living in neighbourhoods with moderate criminal and 'anti-social' activities. Yet an *isolated* significant result such as this, as with the demarcation of the 4th month of pregnancy in the September 11 study, is not necessarily instructive. Further, the difficulty of inferring from these results is that whereas previous confirmative findings of stress and reduced male birth proportion concern acute exposures, design of this analysis is informed by study on the chronic psychological stressors of neighbourhood disorder and deprivation. A closer examination of literature and previous research also illustrates the contradictions associated with varying temporal sex ratio results and uncertainty over the biological mechanisms in reproduction which may or may not be connected to stress responses. It is therefore difficult to sustain the argument that population stress is responsible for the modern declining sex ratio trends

6.4 Maternal Age/ Parental Age

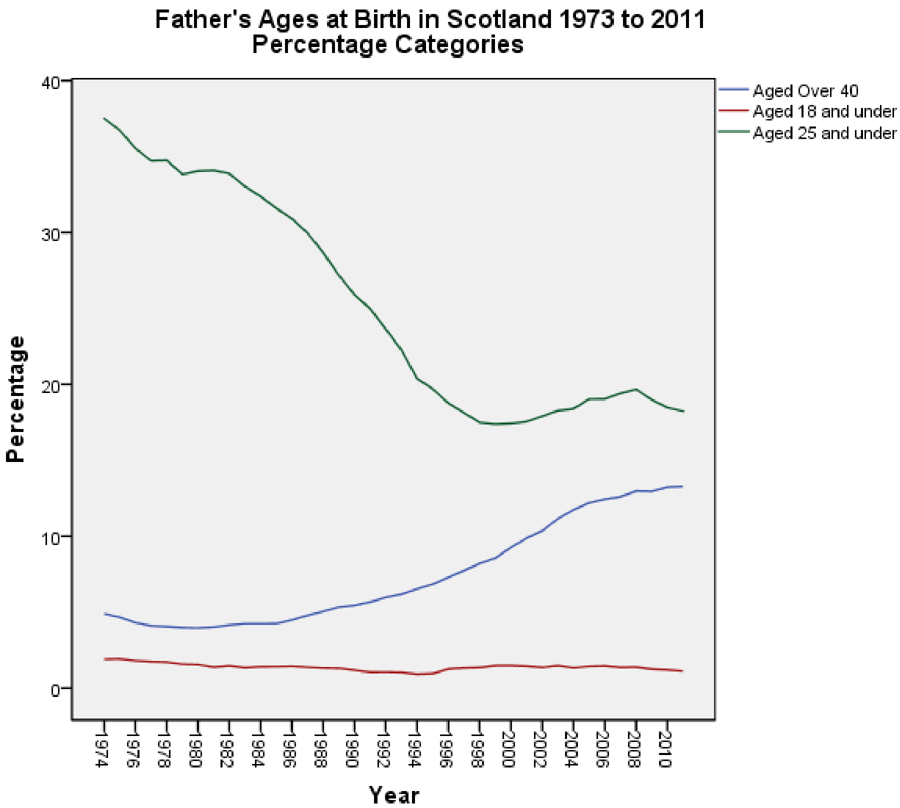
The lack of association in the results between maternal age, measured by percentages of mothers aged 35 and over in a datazone, and secondary sex ratio in the same area, is unequivocal. Pearsons coefficient and Spearmans Rank testing show no correlation whilst stratified analysis (see 5.3.4) reveals no significant or near significant male birth proportion values from the national ratio. On this basis the hypothesis can be rejected but there are important mitigating factors which obscure some of these results.

Firstly, the total numbers of mothers aged 35 or over per datazone over the 2002 to 2010 period is frequently low, meaning many percentages calculated are effectively exaggerated. Secondly, area-based measurement of the two variables is limiting to an extent, as it cannot account for outliers within nominal datazones. For example, multiple datazones which each have one mother aged 35 or over who give birth to a girl are inadvertently missed in the stratified and Pearson's/Spearmans analyses. Thirdly, the Scottish Neighbourhood Statistics data-set only contains maternal age as an indicator whereas previous sex ratio studies also investigate paternal, and both parental age/s. Intuitively, it would be reasonable to assume that advanced maternal age is strongly correlated with older fathers amongst birth couples. An empirical example is given in a combined parental age study on miscarriage (de La Rochebrochard and Thonneau 2002) where over 75% of older mothers had a male partner who was also over 35 years of age. Ostensibly though, validity is slightly weakened without both parental age measurements included in the analysis.

It is difficult then to trace alterations in sex ratio in small areas arising from greater or smaller proportions of older mothers. National statistics for paternal age at birth are available in Scotland over the 1973 to 2010 period and trends are shown in Figure 14. The percentage of fathers over 40 has risen from the mid 1980s of 5% to nearly 13% in 2010. Concurrently the proportion of fathers aged 25 and under has dramatically fallen from 38% in 1974 to 18% in 1998 and then levelling to similar figures up to 2010. There has also been a very small decrease in the percentage of fathers aged 18 and under from 3.2% in 1974 to 2.9% in 2010. From this national projection of paternal age figures it may be easy to conclude a direct correlation with the decline in male birth proportion of births in Scotland, as was illustrated in a number of early ecological studies on parental ages and birth sex ratio (Moran, Novitski et al. 1969; Nicolich, Huebner et al. 2000). Further, a previous study on Scottish national birth data from 1961 to 1972 also established an association with maternal age and small decline in sex ratio when controlling for “social class”, defined by husband’s occupation. Ecological fallacy, however, may be symptomatic of these studies as well as linking the above trends to sex ratio decline.

Although controlling for socio-economic status and parity is undertaken for some of the age-focused analyses this still excludes many of the more recent potential explanatory factors such as pollution or population stress. Adjustment for such variables is also recognised as a limitation in the most recent study confirming the age hypothesis (Matsuo, Ushioda et al. 2009). There is undoubtedly a difficulty arising from an insufficient range of data for many national birth tabulations but it is feasible for future research to design cohort investigations which include measurements of environmental exposure and stress, such as proximity to industrial sites, or General Health Questionnaires. More locally, the Scottish Longitudinal Survey presents an opportunity to address this methodological problem with cross-referencing of sex at birth, age of mother, age of father, mother's occupation, father's occupation, and health status variables. This survey is a 5.3% representative sample of the Scottish population with data commencing from 1971. An advanced analysis of this data-set would be a distinct and complementary addition to research such as this.

Figure 14: Father's Age at Birth in Scotland 1973-2011



(Source*: by e-mail request, Information Services Division – NHS Scotland, 2012* http://www.isdscotland.org/Health-Topics/Maternity-and-Births/Births/)

Contradictions thus arise in concluding from previous research and national statistics.

However there is a unique *utility* attached to analysing parental age as an explanatory variable in reproductive health studies. Age is a straightforward concrete measurement which does not involve estimating exposure based on timing or bio-markers, or proxy quantification with validity weaknesses such as socio-economic status and population stress. This dependable characteristic may partly explain the extensive research and literature on parental age and reproductive health, including other epidemiological subjects. A review of such knowledge and of secondary sex ratio research in Chapters 2 and 3 introduces a number of potentially relevant biological mechanisms to evaluate. A problem for advancing scientific effort in this area though is reconciling whether male or female, or conception based mechanisms, or a combination of these, are responsible for sex ratio alteration and wider reproductive health implications.

A decrease in coital rates amongst older couples was an initial hypothesis promulgated in early studies on secondary sex ratio and age (James 2009). It was proposed that an XX spermatozoa fertilisation (female) was more probable during conception at an earlier time in the female menarche cycle, thus with increased coitus over the whole fertile cycle the chances of a male birth were more likely. Ongoing clinical research though of couples and assisted reproductive technology has not supported such a hypothesis rendering the decreased coital rate mechanism, related to age and/or stress, as questionable. James's hypothesis however, of hormone levels for males and females at conception, testosterone and gonadotrophin respectively (James 2008), may be a valid subject of inquiry given clear evidence of androgen decline in older males (Juul and Skakkebaek 2002). On the subject of singly *male* parameters are two extensive reviews on paternal age, providing detailed evidence on many reproductive functions (Kuhnert and Nieschlag 2004; Sartorius and Nieschlag 2010). Sartorius and Nieschlag

(2010) indicate increasing paternal age as associated with alterations in testicular morphology as well as semen volume and motility, and also cite 5 studies which find age as linked to reduced DNA integrity in sperm. Importantly, these review results accompany other recent scientific debate and research, including (i) TDS and its possible link to endocrine disruption (ii) environmental pollution and associated alterations in sperm DNA integrity, (iii) altered genome imprinting from sperm in the epigenetic post-fertilisation stage and the potential for less male excess reproductive functioning (see 3.3.5) (Boklage 2005).

DNA structural integrity of sperm and its observed deterioration for older men can be linked to two potential mechanisms for propensity for a reduced birth sex ratio. Firstly, altered DNA sperm integrity may have an effect on genome imprinting in the post fertilisation epigenetic environment and consequently alter the ‘normal’ genetic sex determination of male excess. Secondly, two retrospective analyses of pregnancies and couples and a further cohort study have demonstrated increasing risk of miscarriage and fetal death for older fathers (de La Rochebrochard and Thonneau 2002; Slama, Werwatz et al. 2003; Slama, Bouyer et al. 2005) with structural chromosomal aberrations in sperm, including telomeres lengthening (DNA sequences at end of chromosomes) as well as DNA fragmentation, as potentially responsible (Sartorius and Nieschlag 2010). In populations, a greater incidence of miscarriage and the greater propensity for male fetal deaths above the 105:100 sex ratio then leads to a decline in the male proportion of live births. This dual proposition is also pivotal to the environmental pollution ‘sentinel health event’ case, although environmentally mediated genetic damage, rather than endocrine disruption, is presented as the determining influence. The difficulty in sustaining the combined pollution/advanced paternal age thesis is that confirmative results on environmentally mediated genetic damage to sperm are confined to young men (Rubes, Selevan et al. 2005).

It is feasible then that paternal age and the accumulation of environmental toxins, whether by proximal residence to pollution, or long term occupational exposure, could be closely connected to alterations in reproductive health parameters. For this study, results from the spatial analysis of central Scotland do not confirm these new hypotheses (see Maps 7 and 8 showing maternal age categories and air pollution). This is unsurprising given the problem of 'exaggerated' percentages for maternal age in such small areas and the relatively short period, from 2001 to 2010, for which the variables and sex ratios were calculated. The spatial analysis conducted however, demonstrates new methodologies and tools, with the use of area-based datasets and mandatory pollution reporting in GIS formats to investigate male reproductive health determinants, and potentially other environmental and/or social epidemiology. For future designs of studies, data with temporal longevity could be the focus for analyses which could incorporate time series models. There is also the possibility of examining occupational exposures in Scotland through analysis of the Scottish Longitudinal Survey. At present there is very little evidence of combined environmentally and age related effects on sperm quality, such as DNA fragmentation, thus emphasising the need for apposite cohort and cross-sectional studies.

Age and female reproductive/peri-natal health, as summarised in Chapter 4, is extensively documented in epidemiological research and disseminated widely to midwifery and obstetric practitioners as well as pregnancy advice to expectant mothers. In a recent analysis of a 15 year national period, with nearly 1.3 million births examined, adjusted odds ratio for intrauterine fetal death was 3.8 for women aged 45 years or older and 2.1 for the 40-44 years range compared with 1.4 for maternal ages 20 to 29 (Jacobsson, Ladfors et al. 2004). Maconochie, Doyle et al. (2007) identify a 75% increase risk of miscarriage for mothers aged 35 to 39 and fivefold rise for those aged 40 and above. These authors also highlight indicators of stress as associated with increased risk of first trimester pregnancy loss, particularly relating to a traumatic event or stressful job. An intriguing finding for the case-control study in relation to this research is that factors associated with greater risk mirror with potential determinants of male birth proportion identified from the earlier reviews in this study, namely age and stress, whilst similarly no substantial evidence was found for social class, smoking or diet.

The declining proportion of male births explained by advancing ages of parents in industrialised countries therefore initially appears a rational, plausible thesis given the sound biological mechanisms outlined and national data on increasing proportion of older fathers and mothers inversely paralleling the secondary sex ratio trend. When examining regional and sub-regional statistics and small area-based measurement of maternal ages over 35 however, there is little evidence to support the hypothesis. The one exception to this observation are of sex ratio calculations for Health Board Areas for years 2008 and 2010 (see Table 18). Regional data which may provide further insight on the advanced age premise, Table 32 below, shows the cumulative percentage of older mothers (aged 35 and over) for each local authority area in Scotland from 2000 to 2011.

Table 32: Percentage Maternal Age >35 and Median Incomes by Local Authority Area in Scotland.

Local Authority Area	**Percentage first time mothers aged 35 and over: 2000-2011**	**Local Authority Area**	**Median gross weekly earnings for full-time males and females (residence based), 2001-2011 (Mean)**
Edinburgh, City of	18.7	East Renfrewshire	1018
East Lothian	16.7	East Dunbartonshire	992
East Dunbartonshire	16.6	South Ayrshire	952
East Renfrewshire	16.5	Edinburgh, City of	933
Stirling	15.6	Eilean Siar	924
Scottish Borders	14.3	Stirling	919
Perth & Kinross	14.1	Aberdeen City	896
Argyll & Bute	14.1	Aberdeenshire	895
Orkney Islands	13.7	Orkney Islands	882
Eilean Siar	13.7	East Lothian	868
Aberdeenshire	12.8	East Ayrshire	854

Source: Scottish Neighbourhood Statistics (2012) www.sns.gov.uk

The table reveals a clear association with increased social mobility and wealth, and later child-rearing, as the 12 local authority areas containing the largest percentages of older mothers and 12 highest gross weekly earnings for both partners is shown. 8 of the 12 local authorities with the greatest income levels for households in Scotland also contain the largest percentages of first time mothers aged over 35. Sociological research has established shifts in marriage patterns over the last 50 years. In particular, a doubling in the number of women marrying partners more than 7 years older than them, and also women more likely to choose a life partner in their social class (Institute for Public Policy Research 2012) As well as this ‘associative mating’ trend couples are more likely to delay fertility and child rearing for individualistic lifestyle reasons compared with more the communitarian societal/religious mores of 50 years previously. The attainment of financial security is another motive for a delay in parenthood for middle class couples as is partly illustrated in Table 32 with advanced parental age paralleling upward mobility in social class. For secondary sex ratio, however, as documented in the literature review, evidence of class or socio-economic status as a determinant is heavily contradictory. Further, on examining the stratified results for the 20 multiple deprivation categories there is no distinction of a downward trend in sex ratio according to the least deprived neighbourhoods. Assuming the social trend of advanced parental age amongst higher income couples a concomitant pattern expected would be lowering male birth portion in the higher SIMD vigintiles which does not eventuate.

Counter to this argument is the identification of the Lothian Health Region in the Tables 18 & 19 in Chapter 5, as a significant regional contributor to the declining male birth proportion in Scotland for 2008 and 2010. The Lothian Health Region includes the Edinburgh and East Lothian local authority areas, the two highest zones of percentage of mothers aged 35 and over in Table 32. It is also interesting to note East Lothian is one of the local authorities with a low cumulative secondary sex ratio of 0.508 from 1991 to 2010, as well as Scottish Borders (0.506) which has the 6th highest percentage of advanced age mothers. Evidence for parental ageing as contributing to decline in male birth proportion in Scotland therefore varies according to geographical scale, national and regional trends are suggestive whereas 'small area statistics' are not confirmatory. It is unlikely that the increased proportion of older couples conceiving in Scotland is singly instrumental but rather additional to other social and/or environmental determinants. Growth in sperm DNA structural integrity amongst males, embryogenesis changes from altered paternally imprinted chromosomes, greater occurrence of early pregnancy loss and 'preferential' spontaneous abortion of male fetusus, may all be mechanisms which are a *common link* across sociologically related factors, parental age and population stress, and environmental causes, principally industrial pollution. The Maconchie case-control study, in also finding age and stress as linked to increased miscarriage risk, further emphasises a multi-factorial explanation for declining sex ratio trends. The multi-factorial hypothesis of pollution and maternal age tested in this study through overlay mapping is not sustained, however, many of the limitations for use of area-based measurement discussed in chapter 4 (see 4.8) are applicable, particularly as more factors are integrated into such a spatial assessment. The application of GIS techniques and use of ‘area-based’ indicators with pollution measurement is an example though of method which can be adopted to tackle secondary sex ratio and reproductive population health research questions.

6.5 Smoking

High incidence of maternal smoking, measured by women reporting at first ante-natal appointments as smokers in small areas, does not associate with low male birth proportion in Scotland and the minor hypothesis is rejected. This result is further legitimated by the under-estimate of self-reported smoking in deprived areas inherent to SIMD and SPHO measurement documented in recent research (Shipton, Tappin et al. 2009). It also confirms the 5 follow up analyses which repudiate the original Fukuda et al study that established negative associations.

The advantage of the area-based approach to analysing smoking is the comprehensive coverage of all recorded births in Scotland from 2001 to 2009 but there is no dose-response effect formulated based on levels of smoking as only a binary coding of smoking or non-smoking females is measured. Insight, however, is provided by the strong auto-correlation of maternal smoking and multiple deprivation by small area, a reflection of a perennial epidemiological focus on low socio-economic status and poor health status. As discussed previously, multiple analyses of multiple deprivation and sex ratio yield no positive or negative associations; a scattergram (Figure 13) showing a random plot of the two variables is instructive. Further evidence therefore, by considering social class influences, demonstrates no link between maternal smoking consumption and alterations in secondary sex ratio.

Paternal smoking meanwhile is not included in SPHO and SNS area-based statistics and additionally the scoping review notes that rebuttal of the Fukuda sex ratio and smoking study is pre-dominantly confined to analyses of maternal consumption. This gap in health data publication and research is noteworthy as some studies have found deterioration in adult male sperm quality, including volume, concentration and morphology, as attributed to cigarette smoking (Collodel, Capitani et al. 2010). Further, recent studies have demonstrated increased fragmentation in sperm DNA integrity from smoking amongst idiopathic infertile men (Elshal, El-Sayed et al. 2009), particularly for heavy smokers (Gaur 2007). Such findings are qualified though with a meta-analysis establishing contradictory reports of adult male smoking and impaired spermatogenesis, concluding that only a small negative impact may be occurring (Vine, Margolin et al. 1994). Design of reproductive health studies which also include data on *paternal* smoking consumption levels and sex of birth for partners may be beneficial for further elucidation on this subject. It is questionable, however, given the lack of consistent evidence on other reproductive parameters, as compared with paternal age and stress, of any discernible link between paternal and/or maternal smoking and ‘current’ birth sex ratio emerging.

Alternatively, pre-natal exposure to smoking and imbalances in the acceding generation’s sex ratio may be a hypothesis to explore in future investigations. An important contribution made in a review by Richard Sharpe, on joint environmental and lifestyle impacts and intra-uterine exposure and consequent impaired spermatogenesis in the adult male (Sharpe 2010), is highly relevant to this subject as well as for other potential explanatory factors. Reproductive biology confirms that foetal testosterone levels are dependent on Sertoli cell proliferation and any irreversible disturbance of such a mechanism during testis development in the womb is likely to affect eventual adult sperm production. Sharpe (2010) cites a number of large studies showing up to 40% reduction in sperm counts amongst men whose mothers were heavy smokers during pregnancy. An additional and more recent cross-sectional study indicates associations with not only impaired testicular function but also earlier onset of pubertal development.

Significantly for this thesis and its multi-factorial perspective to secondary sex ratio determination, the Sharpe review details other pre-natal exposures including dioxin pollution (citing a Seveso study), beef consumption, diesel car/exhaust fumes, sedentary lifestyle and obesity. Therefore two pervasive conceptual arguments potentially envelop the smoking and sex ratio hypothesis. Firstly, an intra-generational process of cause and effect, pre-natal exposure and consequent altered reproductive health in adulthood may explain the declining male birth proportion phenomenon. Secondly, pre-natal smoking exposure may be combined with other environmental insults or health-related behaviours to contribute to secondary sex ratio changes in industrialised populations. For this study's results, the skewed downward sex ratio trends for regional Scotland are observed over a long period, 1973 to 2010, thus modelling potential inter-generational population events. The difficulty, however, in sustaining Sharpe's arguments if transposing to sex ratio decline is that smoking and the lifestyle factors described, such as obesity and lack of exercise, are easily translated to the population groups experiencing multiple deprivation, and examination of this key health determinant in Scotland is strongly inconclusive on male birth proportion. This important dimension to secondary sex ratio debate is discussed in the next section. Meanwhile, inter-generational analysis of birth sex ratio may offer insight into possible sentinel reproductive health events across populations. Studies previously conducted include those on the DES male and female cohorts, whose mothers were prescribed artificial oestrogen during pregnancy, with no alterations in sex ratio reported (Wise, Palmer et al. 2007; Wise, Titus-Ernstoff et al. 2007). Longitudinal data-sets are potential tools for such inquiry as well as the data-mining techniques currently in vogue in epidemiology.

6.6 Socio-Economic Status/ Multiple Deprivation

In contrast to other factors and indicators analysed, significant male birth proportion values are established from stratification by Scottish Index of Multiple Deprivation vigintiles for 2006, though there is no discernible uniform trend in their distribution across the 20 categories. There is also no overall correlation in small areas secondary sex ratios in Scotland however, either by Pearsons or Spearmans Rank method with any deprivation type (e.g. health deprivation, crime deprivation) or by a formulated variable of normal distributed multiple deprivation values (see 5.3.2). Perhaps of greatest importance is the ‘spike’ in sex ratio for the highest SIMD vigintile, the least deprived, and thus most advantaged, population group. The category 1 cohort experiences very high levels of wealth and income, superior housing type and is least exposed to neighbourhood crime. This result would initially seem to reinforce the evolutionary psychological theory of Trivers-Willard described in Chapter 2 (2.3.2.1). There is however no gradated trend across the other 19 vigintile groups of a Trivets-Willard pattern, that of low sex ratio for those residing in the most socio-economically disadvantaged and health deprived areas and high sex ratio for residents of least deprived, richest population areas. It is also difficult to corroborate the hypothesis with these results as the 2nd least deprived group displays a *low* male birth proportion figure, thus opposite to the predicted effects of Trivets Willard. Moreover, as per the neighbourhood stress results, a loose trend which does emerge is more of a 'book-end' effect with a significant increase in sex ratio also for the 2nd most highly deprived vigintile mirroring the highest income group's 'spike'.

As for a large content of the secondary data analysis on socio-economic status and testing of the Trivets-Willard hypothesis, results in this doctoral study are not affirmative of socio-economic determinism and birth sex. Even at different geographical scales, for example by parliamentary constituency, the predictions from such a hypothesis would see areas such as Glasgow Shettleston, Greenock & Inverclyde and Dundee City East identified and is not reflected in the results in Chapter 5. In parts of the West of Scotland there are some of the highest concentrations of poverty in the U.K. and Western Europe which are delineated and documented by the SIMD area-based measurement described in Chapter 4 (4.6.2). A distinct lack of evidence for downwards skewing in such areas therefore further undermines the Trivers-Willard proposition.

There are a number of methodological and conceptual problems associated with this hypothesis and evolutionary psychological theory on the human secondary sex ratio. Firstly, definition of 'socio-economic status' is very limited in many studies, sometimes only extending to occupational or educational status of father, whereas deprivation, used in this analysis, is a multi-factorial measurement of socio-economic status. Further, although multiple deprivation data exist in the area-based format compared with individually tabulated records, significance established in results may be more important than very small adjustments in sex ratio with large ecological studies of national or regional populations.

The environmentally defined nature of the statistic means that socio-economic and health status can be determined by where a person lives and therefore correlations are frequently found with deprivation and other health outcomes/measures e.g. low weight births, cardiovascular disease. Secondly, unproven conjecture on sociological phenomena persists amongst proponents of the Trivers-Willard hypothesis and ancillary evolutionary psychological theory. Statements on potential socio-economic factors affecting sex ratio tend to be presented as assumed ontological fact rather than supported by concrete data and/or sociological theory on structure or agency. This particularly surrounds speculation on the Second World War sex ratio rise, commonly referred to as confirming evidence of the hypothesis. James suggested increased frequency of coitus occurred amongst returning soldiers and their partners during a compressed time of leave and together with the timing of conception hypothesis and a Trivers-Willard 'good condition' effect, more sons are likely to be born in the population. Whilst such a scenario is intuitively plausible James does not provide sound historical or sociological data, sourced perhaps from surveys or interviews, to validate such a claim.

Unsubstantiated conjecture is also offered by Valerie Grant in her otherwise informative book, 'Maternal Personality, Evolution and the Sex Ratio', which presents evidence and arguments for the ‘maternal dominance hypothesis’ (Grant 1998). The author describes social scientific research which ignores key biological theory on reproduction as ‘reprehensible’, however then in turn wildly speculates on spikes in sex ratio in a Central Scotland study as attributed to ‘women caring for the sick’ and thus a collective female testosterone rise, and explains a significant yearly drop in the Irish sex ratio by ‘tough young women’ leaving Ireland to undertake abortions in the U.K. Once again there is no data or evidence to support such claims; it is an example of the far too frequent grand speculation that can accompany socio-biological perspectives on secondary sex ratio.

Reaffirmation of the Trivers-Willard hypothesis thus relies on the following; (i) isolated large U.S. demographic analyses which show minor adjustments in male birth proportion restricted to specific variables illustrative of parental investment or socio-economic status i.e. married, better educated mothers (Freese and Powell 2001; Almond and Edlund 2007) (ii) the U.S.A. II World War sex ratio trend (the Scotland data however, Figure 3, shows a continuous rise in sex ratio well before and beyond the war), and (iii) Kanasawa’s selective sex ratio studies on odd cohorts such as ‘tall, returning soldiers’, ‘violent men’ and ‘beautiful daughters’ which contain confounders disproving Trivers-Willard and severe shortcomings in validity (Kanazawa 2006; Kanazawa 2007; Kanazawa 2011). The Trivers-Willard hypothesis was first propagated in 1973 and there has been much academic energy expended on this theory with a very limited number of confirmative results. Due to the problems outlined above and association with socio-biological determinism (many TW articles appear in the journal Social Biology which superseded Eugenics Quarterly), it may explain the lack of a thorough contribution to secondary sex ratio inquiry from sociologists. Further, the declining proportion of male births in certain national populations is also not satisfactorily explained by Trivers-Willard research, although Grant has proffered a suggestion from ‘maternal dominance’ theory that in Western societies the exit of ‘son producing’ dominant females from the home environment and child-rearing into the workplace, and thus remaining childless, results in less males being born (Grant and Yang 2003). This is not evidenced by any concrete data and even assuming the existence of ‘dominant’ and ‘non-dominant’ women, a sufficiently large enough difference in birth rate to affect a national birth sex ratio is highly unlikely.

This study’s conclusion on the dismissal of the Trivers-Willard hypothesis does not however remove the need for sound epidemiological inquiry of reproductive health in multiply deprived areas. Sharpe’s detailed distillation of modern ‘lifestyle’ factors and their effects on male reproductive health emphasise the importance of investigating areas where adverse health indicators such as smoking, obesity and alcohol intake are greatest. There is extensive documentation of the existence of these factors in the most deprived neighbourhoods of cities and towns and therefore the analysis of SIMD provides valuable insight. If differences in sex ratio according to socio-economic status were to emerge it could also present the possibility of a dual effect of pollution and deprivation in many geographical areas. This would be consistent with previous ‘environmental justice’ research undertaken, though results indicate - both for the SIMD vigintile tables and industrial pollution and deprivation mapping - no such auto-correlation and skewed sex ratios. For the spike of vigintile 1, excellent maternal health, very low exposure to stress, and adoption of reproductive technologies, as documented in the U.S. amongst the wealthiest in society (Hvistendahl 2011), may be potential explanatory hypotheses to explore further in the future. Indeed, a rare focus for social epidemiology on more privileged groups in society may be instructive for reproductive health research.

6.7 Environmental Pollution

The geographic distribution of regional and sub-regional birth sex ratio trends in Scotland is moderately indicative of pollution type effects on reproductive population health. A closer examination of the areas affected in Central Scotland, which involves GIS mapping overlays, shows small areas of low sex ratio at the 5%,10% and 25% significance level to be concentrated in, or close to, areas where data sets of endocrine disruptor air pollution have been produced. Further, calculations of annual post-war sex ratios, from 1944 to 1973, reveal an urban/rural dichotomy with non-metropolitan areas, pre-dominantly distant from sources of industrial pollution, defying the national declining trend in displaying a slightly upward trajectory. The results outlined tend to corroborate observations of a sentinel reproductive health change, though not necessarily tied exclusively to the environmental endocrine hypothesis as the proposition of environmentally mediated genetic damage to spermatozoa is also pertinent. Such findings are flagged because of the range of geographical resolutions at which trends and dual concentration is observed, and the longevity of sex ratio change displayed. As an example, in Falkirk and Grangemouth isolated neighbourhood areas (datazones) are skewed downwards for male births from 2002 to 2010, whilst in measuring at a larger scale the local Parliamentary Constituency of Falkirk West shows significantly cumulative low male birth proportion for a slightly different time period (1999 to 2007). For the regional scale, Falkirk contains the 3rd lowest birth sex ratio (by Z score) of 32 local authorities in Scotland over a longer time period (1991 to 2010) whilst in Tables 8 and12 the breakdown of secondary sex ratio by larger regions (health board areas) documents the key downward influence on the national ratio by the Forth Valley local health authority over the apposite 1973 to 2010 timeframe. The 'mono-geography' problem of over-interpreting results from a single areal scale is thus avoided to a limited degree by extensive geographical coverage in this analysis. This problem, however, frequently a criticism of environmental justice secondary data research, usually concerns *multi-variate* modelling and/or mapping at one scale which also reflected the 2nd stage of the design of this study. The qualitative difference in this thesis is the series of additional uni-variate trends of sex ratio over significant time periods which offer substantiation of multi-factorial analysis, and further elucidation of research questions.

A crucial methodological challenge which also must be addressed for these results and the subsequent interpretations is evading *ecological fallacy.* As has been discussed in appraisal of previous studies there is danger in over-interpreting associations which result from analyses or post causative events (e.g. natural disasters, economic depressions) as a confounding factor(s)/event(s) may be responsible. Attempting to establish relationships between two or more sets of data through statistical testing and inference is the foundation of positivist methodology. A statistically significant result for any positive or negative correlation between data-sets is suggestive of cause and effect in both natural and social science. As discussed in Chapter 4 (4.8) there are a number of unique characteristics to the dependent variable of 'secondary sex ratio by area' which render the probability of establishing associations with socio-economic and health-related area indicators unlikely. The focus of this research has been to provide an extensive portfolio of temporal trends, analysis and spatial projections in order to 'map out' secondary sex ratio in Scotland. This geographical emphasis, incorporating area-based measurement, differs from a standard positivist and epidemiological approach and offers an alternative method of addressing the birth sex ratio question.

An instructive feature of the GIS spatial analysis shown in Chapter 5 is the delineation of low sex ratio neighbourhoods near to certain sources of industrial endocrine disruptor air pollution which can be closely examined. There are two particular localities which require highlighting as both are potentially important signifiers of a historical legacy of pollution in Central Scotland. The first case is the Bonnybridge area, to the south west of Falkirk (see Fig 34) which displays a significant excess of female births, thus replicating findings from Williams, Lawson et al. (1992). For the Williams study The FK4.1 postcode sector resulting sex ratios were 0.472 (z statistic 2.16) and 0.474 (z statistic 2.04) for 1975-1979 and 1980-1983 respectively. This 'at-risk' area was in close proximity to the ReChem waste incineration plant near Bonnybridge, an emitter of dioxins, PCBs and polycyclic aromatic hydrocarbons (PAHs) which subsequently closed in 1984. Emissions from burnt waste at this site remained a high-profile environmental controversy in Scotland, with nearby incidents of infant eye defects requiring a comprehensive morbidity investigation by the Scottish Office (Scottish Home and Health Department 1985) and an unsuccessful legal case pursued by local farmers following the loss of their cattle. Williams, Lawson et al. (1992) also cite an agricultural report on animal health problems in Bonnybridge and Denny which established 'comparatively high concentrations' of PAHs in surface soils of the 'at-risk' postcode districts (FK4 and FK6). Given the lipophilic properties of PCBs and PAHs there is a potential exposure risk of soil contamination in this locality with continued skewed sex ratios a possible local sentinel indicator of such risk.

As identified in Chapter 5 (5.4.2), a locality of concern is East Cambuslang in South Lanarkshire, a population growth area where a significant downward skewing of sex ratio is occurring. Similar to Bonnybridge a case of potential environmental hazard emerged in the 1990s with the legacy of contaminated land from the UK's largest chromate works (Whites Chemical) in Rutherglen (Eizaguirre-Garcia, Rodriguz-Andres et al. 1999). Following residents' concerns due to local cases of leukaemia, epidemiological investigations were undertaken with null findings. Since this time extensive remediation of contaminated land, particularly in South Lanarkshire, Glasgow and North Lanarkshire, has been undertaken in order to release land for residential and commercial brownfield development. This a cornerstone of urban regeneration strategy in Scotland, facilitated by the Scottish Government, local authorities, EU agencies and private developers.

The regional concentration of reduced sex ratios within local authority areas such as South Lanarkshire (see Table 21) and parliamentary constituencies, including those outside the GIS study area such as Paisley North, may be 'sentinel' alerts of heath risk from the legacy of heavy manufacturing industrial pollution in Central Scotland and a transition to redevelopment of such environmentally degraded land. Most recently, ill health has been reported by residents at a Motherwell housing estate in Lanarkshire which was constructed on land for a munitions factory (Miller 2012) Litt, Tran et al. (2002), on investigating urban brownfields in Baltimore, recommend improved co-ordinated environmental health and urban planning with re-development and public health screening to be undertaken before remediation of land.

Cancer epidemiology statistics for Scotland are also noteworthy with regard to sentinel indication of reproductive health and the aforementioned geography. For the period 2004-2008 the crude rate of cancer of the testis (per 100,000 person-years at risk) for Lanarkshire and Forth Valley health regions was 9.9 and 8.8 respectively and based on age-standardised incidence, on the upper confidence interval of prediction, are the two highest values in Scotland (NHS Information Services Division 2008). This compares with a smaller 7.7 crude rate for cancer of the testis in Greater Glasgow and Clyde, a health board area which has significantly more cancers of all types and is recently identified as possessing specific ‘Glasgow effects’ which contribute to poor population health (Walsh, Bendel et al. 2010).

In the context of male reproduction it is also important to emphasise the two robust local studies which document sperm quality decline in the last 40 years, one undertaken in Edinburgh and another in Aberdeenshire (Irvine, Cawood et al. 1996; Sripada, Fonseca et al. 2007). Hence in accounting for descriptive epidemiology in Scotland and broad population health indicators such as that on secondary sex ratio shown in this thesis, there is gathering evidence that testicular dysgenesis syndrome with an environmental aetiology exists locally. Both environmental hypotheses make reference to the epidemiology of testicular cancer and geographical differences in sperm quality in industrialised countries with recent commentary examining pollution and genetic, and pollution and health/lifestyle compounding effects (Joffe 2010; Sharpe 2010). Positioning these findings with either the environmental endocrine hypothesis or environmentally mediated genetic alteration is not a crucial objective for this research. In common with both hypotheses, however, the mediation of chronic long-term exposure of pollution, potentially over generations, and including mixing of low doses of chemicals, requires additional understanding from on-going scientific research.

Further, the inclusion of secondary sex ratio monitoring with new methods of environmental assessment, on cumulative impacts from pollution, is also worthy of consideration (Alexeeff, Faust et al. 2012; August, Faust et al. 2012).

The *inter-generational* genetic sperm damage thesis proposed by Joffe is of substantive interest for results of this study, particularly the length of the period examined for regional health boards, 1973 to 2010, and Forth Valley displaying the foremost ‘leverage’ of sex ratio. The recent Teplice research in the Czech Republic which tests sperm chromatin assays on young men chronically exposed to sulphur air pollution finds DNA sperm fragmentation to be significantly elevated (Rubes, Selevan et al. 2005). This suggests that chromosomal sperm instability and thus alterations to male reproductive parameters are not confined to determinants such as current exposures to the endocrine disrupting chemicals already documented and included for the spatial analysis modelling. Interaction of both paternal age and environmentally mediated inter-generational transference of chromosomal impairment is potentially an additional hypothesis to explore.

The central region of Scotland has been the key area for interpreting results, however, significant trends have also emerged from the south-east and southern regions. The significantly skewed low male birth proportion in Roxburgh and Berwickshire Parliamentary Constituency area from 1999 to 2007, where female births outnumber male births, is important. Additionally, the inverse distance weighting map highlighting low sex ratio distribution which continues beyond this constituency and onto the Dumfries and Galloway region is noteworthy, particularly as it largely covers the Solway-Tweed river catchment. A tentative hypothesis for such an unusual pattern is possible exposure from water pollution in this region, particularly given the environmental pollution problems for this catchment requiring management by SEPA (Scottish Environmental Protection Authority 2007). This may raise issues of agricultural runoff in water courses, silage storage, and sewerage treatment in the area, together with possible exposure routes of recreational fishing as per the Great Lake fishermen families' studies.[2]

Finally, the interaction of environmental pollution, in the form of endocrine disrupting air pollutants, and multiple deprivation which signifies poor health status by proxy, is not demonstrated in this study. Low sex ratio skewing does not transpire in deprived areas of polluted localities in Central Scotland (see Maps 5 and 6). This is not surprising given the results on SIMD vigintile categories with an upwards direction in male birth proportion for the most deprived groups. The methodological challenge of the 'mappable area problem' in spatial epidemiology, particularly for environmental justice research, is also relevant to this analysis. Applying a different geographical resolution to the multi-factorial analysis may have yielded dissimilar results, particularly for the South Lanarkshire and North Lanarkshire regions where highly deprived areas are particularly prevalent. Incorporation of pollution measurement by other media types, in particular land contamination, may also affect final findings from this type of research. An endocrine disruptor exposure matrix was attempted in this study, including proxy measures of air, water and land pollution, however, establishing weightings and thus exposure magnitude was extremely problematic and jeopardized overall validity for the research.

[2] Appendix F is a map from the SEPA Water Management report on Solway-Tweed basin. An environmental health hazard may exist in the Berwickshire area in accounting for the sex ratio statistics and IDW mapping from this study

6.8 Limitations

As previously discussed (Chapter 4) there are important limitations implicit in this type of research, common to analyses of secondary area-based data-sets. Significant methodological issues include the 'moving target' problem for exposure measurement or mobility of persons and households out-with the subject areas, the imprecise nature of area-based measurement vis-a-vis agglomeration of individual data, and interpreting findings from single geographical resolutions of spatial data. Some of these issues were tackled to a limited extent in this research. A large variety of geographical resolutions have been adopted, including national, urban/rural, regional, sub-regional, and small areas (agglomeration of neighbourhoods). Multivariate analysis though is confined to small areas with birth sex ratio values for other larger areas providing important insight. This breadth of coverage in fact strengthens the validity of the area-based approach, as invariably individual data formats are at risk for disclosure. This is particularly the case with small area statistics in Scotland where indicators are configured in order to avoid the disclosure problem e.g. weight of prescriptions for depression and anxiety. Despite all variables not being analysed across the areal types, the birth sex ratio spatial-temporal review offers not only further findings but also qualification of small-area results. Addressing the population mobility issue is more problematic although the shortcoming is explicitly recognised which is not a consistent admission for population health research using area-based data. The calculation of cumulative sex ratios across larger regional zones once again offers limited remedy given there is less probability of in and out migration of households the bigger the areas become.

Despite some current inherent weaknesses to area-based statistics, this is how national public health data is constructed in Scotland, and restricting secondary analysis of birth sex ratio to individually based socio-economic and health-related statistics, would most likely mean only Sweden, Denmark and Norway were investigated. The Scottish Longitudinal Survey, a 5% representative sample of the Scotland's population from 1973, with cross-tabulation of similar variables possible, could be another avenue for inquiry. Further, the pioneering method of data mining algorithms in public health is similarly applicable. The crucial advantage of utilising geographical area-based measurement is that it facilitates a *comprehensive* inquiry, both spatially and temporally, of secondary sex ratio change in national and regional populations, rather than by acute events or selected cohort groups.

In conclusion, historical and regional trends and localised skewing of sex ratio is commensurate with other studies which link male birth proportion decline with air pollution (Williams, Lawson et al. 1992; Mackenzie, Lockridge et al. 2005). Further, cancer epidemiology statistics for Scotland also show a high disproportionate share of testicular cancer diagnoses in the Lanarkshire and Forth Valley Health Regions. Conflating these reproductive health signifiers however is exercised with caution given the multiplicity of potential factors and exposures involved. Commentators have emphasised the danger of over-interpreting easily accessible sex ratio data to then report fallacious associations. Sharpe (2010) in reviewing environmental and health-related determinants of spermatogenesis both in its foundations at the peri-natal stage, and in adults, illustrates the need for reproductive biologists to investigate multiple effects. This population health study replicates such an approach, whereby the non-invasive, universal measure of secondary sex ratio may serve as an instructive reproductive health marker through inter-disciplinary research praxis.

Chapter 7: Conclusion

7.1 Introduction

Elucidation of the human secondary sex ratio phenomenon for this study has involved two reviews, and extensive temporal, secondary data and spatial analysis in Scotland. Discussion in the previous chapter attempted to review and synthesise core results from these analyses with key epidemiological and public health related work. Key findings which emerge include the suggestion of sentinel reproductive health events, namely chronic low dose pollution risk for downward birth sex ratio alteration and a conflated sociological trend of parental ageing. Evidence of *national* secondary sex ratio decline in industrialised countries mediated by health-related factors, such as smoking and stress, and/or health inequalities measured by deprivation, is inconsistent. As detailed in Chapters 4 and 6 there are limitations attached to the methodology and materials /tools adopted in this study. These shortcomings are discussed together with implications of the study results for present health and environmental policy agendas and future avenues for research inquiry.

7.2 Endocrine Disruption

Science on endocrine disruption is pivotal to the scope of this study and for future research. Since the original Wingspread Consensus statement in 1998, the possible effects of endocrine disruptor chemicals is now receiving significant attention from national and supra-national scientific and health bodies. The potential effects on human health remain controversial. For example, on the basis of precautionary principle and extensive lab-based research, Bisphenol A has been banned from use in the manufacture of baby bottles in some US states and in France. A most recent meta-analysis of 150 BPA exposure studies, however, argued that the concentrations are likely too low in humans to cause oestrogenic effects, even assuming the endocrine disrupter ‘low dose’ effect model. In turn, these results are disputed by other scientists as counter-factual because biological activity of Bisphenol A is not modelled or accounted for (Environmental Health News 2013). In 1995 the U.S. federal government appointed the Committee on Hormonally Active Agents in the Environment subsequently experienced major difficulty in reaching a cautionary consensus position on endocrine disruption amongst scientists. A current WHO report is more equivocal stating that ‘trends indicate an increasing burden of certain endocrine diseases across the globe in which EDCs are likely playing an important role, and future generations may also be affected.’ (World Health Organisation 2013).

As for any scientific knowledge on pollution effects, establishing a definitive causal link between endocrine disruption and human health is problematic. The human population is heterogeneous and subject to multiple exposures, thus comparisons can only be made between more and less-exposed groups (Howard 2001). Inaccurate exposure assessment is encountered even with bio-assays and complex mixtures of chemicals affecting populations are almost impossible to model (Schell, Burnitz et al. 2010). From an environmental science perspective the modelling of endocrine disruptor air emissions for this study is manifestly rudimentary. Approximations are made from estimation and models presented in local environmental impact assessments for proposed waste incinerator facilities. The incorporation of a Gaussian plume distribution model taking account of the height of the discharge point (stack height), prevailing wind, underlying topography in addition to the quantities of endocrine disruptor emissions for each point in Central Scotland would greatly advance exposure assessment and local environmental monitoring. In addition, data collection could involve soil and air sampler testing for airborne contaminants, including concentrations at automatic monitoring stations for selected endocrine disrupting chemicals to validate the model.

GIS techniques can also improve exposure measurement and environmental modelling though this is dependent on the quality of data sourced. Data protection and commercial confidentiality, however, may obstruct specific SEPA monitoring and discharge records being obtained. A shortfall for the present study is also that endocrine disruption via other media, such as land and water as well as food, drink and health–related exposures, is not projected in the spatial analysis. Extending modelling to include pollution sources from water, wastewater and land would involve careful assessment of potential routes for human exposure. Further, given this study's results and possible deleterious health effects increasingly documented in scientific study a full environmental audit of endocrine disrupting chemicals in Scotland is recommended. Inevitably such an exercise is unlikely to be exact and all-inclusive, however extending capacity for monitoring endocrine disruptor pollutants within Scotland is warranted.

7.3 Advanced Statistical Methods

Future study designs on potential endocrine disruptor exposure and reproductive health outcomes, such as sex ratio, should also incorporate more sophisticated geo-statistical analysis. Map 1, showing inverted distance weighting of sex ratio distribution in

Scotland is an example of such an approach, facilitated by either clustering analysis, spatial-scan statistics and kriging. It was not possible to use such tools in this study. This was partly due to the 'instability' of the 'birth sex ratio' dependent variable, as discussed in 4.8, with simple correlations unobtainable and thus regression models unable to be extrapolated. Geographically weighted regression is a geo-statistical method which can potentially be applied to other birth and/or reproductive health indicators and determining factors e.g. low weight births and NO^2, deprivation and maternal smoking 'datazone' variables (see 6.3). Multi-level modelling is also a sound method for analysing interactive determinants such as pollution and parental age or deprivation and pollution. Finally, poisson regression is another alternative with modelling of a small count dependent variable which for this subject would be number of male births in small areas. In summary there is a suite of geo-statistical and advanced statistical methods which can be employed on associated area-based data, thus providing ample opportunity for advancing scientific understanding of environmental effects and/or sociological influences on reproductive health.

Forensic analysis of Scottish Longitudinal Survey data is another research project to consider. The survey is a 5% representative sample of Scotland's population from 1973 to now, with a very low attrition rate. Data linkage is across a large number of demographic and socio-economic variables, including birth sex and paternal/maternal occupation and age. A retrospective ecological study of approximately 120,000 births is therefore feasible and by including the England and Wales Longitudinal Study (1% sample from 1971) the population size could be considerably expanded. A limitation though is that potential inter-generational exposure cannot be tested despite longitudinal coverage, as sex at birth for cases' progeny are not recorded.

7.4 Cohort Recruitment

The need for epidemiological inquiry over multiple generations of inter-uterine exposure and the growing evidence of endocrine disruptor effects presents a strong argument for developing specifically related cohorts. Inter-generational inquiry could isolate mechanisms of biological/gene transference and possibly the initial environmental teratogenic damage which mediates future reproductive health parameters. Recruitment would likely involve prospective parents and follow their offspring through the lifespan to adulthood and the reproductive period. A 'cycle' of biomarkers could potentially include:

(i) pre-birth parental serum samples of reproductive hormones, PCB congeners and other ED concentrations;

(ii) pre-birth father's semen quality measurements (including DNA fragmentation index);

(iii) sex of birth and if male, incidence of crytorchidism or hypospadias;

(iv) son/daughter puberty development markers;

(v) son/daughter early adulthood serum samples a.p. (i);

(vi) son semen quality measurement; and

(vii) return to (i).

The feasibility of constructing such cohorts may be questionable given ethics of inter-generational recruitment and likelihood of attrition occurring for the son/daughter component. These, however, could be similar to the smaller 'DES exposed' and 'Sweden Fishermen' cohorts whereby persons are sourced according to known exposure type e.g. DES, or heavily-exposed occupation and/or locality e.g. Swedish East Coast

Baltic sea area. Couples attending fertility clinics could be recruited or an area-wide campaign in a potentially affected region could be instigated, such as the Forth Valley or Lanarkshire in Scotland. As demonstrated in this study identification of sex ratio skewing in regions or localities could assist in geographically demarcating cohort recruitment areas. Areas selected would also correlate with other reproductive parameters, such as increased testicular cancer or cryptorchidism incidence, thus demarcating potential local sentinel reproductive health change.

7.5 Sentinel Surveillance in Scotland

There is now reasonable evidence from sentinel trends that some regions in Scotland may be experiencing deterioration in male reproductive health. Two robust semen surveys, conducted in Edinburgh and Aberdeenshire, reported declines in sperm quality amongst male donors. (Irvine, Cawood et al. 1996; Sripada, Fonseca et al. 2007) Further, testicular cancer and cryptochordism in the last 30 years have also increased sharply according to epidemiological studies. The secondary sex ratio observations for this study provide accompanying evidence to these signifiers of testicular dysgenesis syndrome in Scotland.

In a recent editorial for the International Journal of Andrology reproductive biologists speculated on a 'crucial tipping point' that may have been reached of below 'threshold' sperm counts in the male population which lead to greater human infertility (Andersson, Jorgensen et al. 2008). Experts have also recommended 'standardised surveillance' of sperm quality is undertaken given further robust studies are coming forward with findings of semen quality decline, including for Finland where previously results were unremarkable (Nordkap, Joensen et al. 2012; Rolland, Le Moal et al. 2013). Spatial and temporal analyses of birth sex ratio incorporating GIS tools, as demonstrated in this study, could potentially supplement such monitoring.

As stated in discussions here, interpreting the significance of the male birth proportion needs to be done cautiously given the multi-faceted nature of sex ratio determination. Conclusions from findings of this wide-ranging study, including both review and analysis, secondary sex ratio surveillance would be most appropriate as a supplementary indicator to 'concrete' epidemiological data on reproductive health e.g. testicular cancer incidence, DNA sperm fragmentation. Up to the year 1949, the Registrar General's Annual Review published by GRO (Scotland), documented national birth sex ratio including a comparative commentary on ratio figures for antecedent years. This could be reinstated annually, including breakdowns for regional figures and temporal trends to monitor any further decline.

7.6 Health Interventions

In addition to outlining prospective research emerging from this study there may also be potentially important health interventions available which merit consideration. Although this health study is pre-dominantly exploratory in scope and does not examine specific pathology or disease states, the observance of a sentinel reproductive health event in Scotland is significant. Such events are defined as changes in population health which arise from avoidable factors and therefore necessitate public health intervention (Davis, Gottlieb et al. 1998). This study has identified such likely factors as endocrine disruptor exposure, advancing parental age and to a lesser extent, population stress, which may contribute not only to deterioration in male reproductive health, particularly from industrial pollution, but also affect couple fertility more generally.

A public health strategy in Scotland and the rest of the UK directed at ameliorating infertility, most particularly in andrology, is recommended. A public awareness campaign on improving the health of partners during reproductive stage of life could feature as part of such a strategy. An excellent example of appropriate material is an information booklet published by Andrology Australia (Appendix C) which advises men on how to look after their sperm. This type of health information could be targeted towards a young demographic by making it available at gyms, G.P. surgeries, and colleges or possibly even at marriage registry offices. A principal objective for such dissemination would be to raise awareness in the community on reproductive health, more particularly on the male fertility role and good quality sperm compared with more prominent educative material on female physiology and peri-natal health. Health promotion at schools could also be initiated with teenage males a target group for education on fertility and andrology. There may also be a case for baseline measurements of sexual development of adolescents in schools if further evidence of endocrine disruptor effects is substantiated, as per recent Belgian reports (Den Hond, Dhooge et al. 2011).

Environmental health interventions are also required with greater integration between environmental monitoring and public health and local midwifery practice. Inclusion of sex ratio monitoring with new methods of environmental assessment which incorporate cumulative health impacts from pollution is feasible (Alexeeff, Faust et al. 2012; August, Faust et al. 2012). This could also involve reciprocal working between environmental scientists and local medical and public health teams exchanging knowledge on specific endocrine disruptor pollutant releases or hazard and incidences or clusters of adverse reproductive health outcomes. Further, health assessments and accordant planning prior to remediation of contaminated land for urban brownfield development is strongly recommended (Litt & Tran 2002). Finally, in adopting the precautionary principle, statutory pollution controls and enforcement strategies that may remove or reduce substances/processes linked to adverse reproductive effects is justified. On March 14th 2013, the European Parliament recommended endocrine disrupting chemicals to be listed as 'substances of very high concern' (SVHC) for existing REACH regulations. This requires companies in member states to apply for authorisation of SVHCs for particular uses or production processes. New UK and Scottish environmental policy, monitoring and enforcement will need to be instituted which addresses environmental reproductive health protection.

7.7 Male Birth Proportion – Over-Interpretation or Signifier?

A spatial-temporal investigation of the human secondary sex ratio in Scotland, together with extensive examination of related research, offers tentative confirmation of Davis's sentinel health hypothesis on environmental influences. Sociological factors, in particular the delay in child rearing amongst parents in many industrialised countries, is likely to be also implicated. The sex ratio phenomenon often receives media attention. Adoption of sex-selective reproductive technology and the evolutionary hypothesis of dominant females are popular features for journalists (Telegraph 2009; Adams 2012). The problem with such reports is that skewing of sex ratio and stated likely causes can be assumed as accepted science. A pertinent academic commentary emphasises the danger of over-interpreting easily accessible sex ratio data to then report fallacious associations (Bonde and Wilcox 2007).

Advanced reproductive technology and increasing peri-natal medical interventions in Western countries, however, do represent gaps in knowledge on the research subject. Recent scientific journalism on the serious demographic imbalances in Asian societies as a result widespread sex selective abortion practice, partly catalysed by U.S. exported birth control policies in 1970s, also documents ‘girl selection’ occurring amongst the super-wealthy in America (Hvistendahl 2011). Research is also scarce on potential effects of induced abortion trends in various countries, with adjustments in sex ratio feasible from pregnancy terminations associated with medical detection of chromosomal abnormalities in the fetus, proportionately higher in males for Down’s Syndrome (Mutton, Ide et al. 1993). The list of factors potentially responsible for male birth proportion change is thus not finite. Further, the exact mechanism of sex determination at conception and epigenesis is not known and without ethically prohibitive human experimental studies unlikely to be understood.

In conclusion therefore, male birth proportion decline in Scotland and elsewhere, *in and of itself*, is not instructive when considering the many aforementioned cautionary statements in this study. However, historical and regional trends and localised skewing of sex ratio downwards, commensurate with air pollution data and a disproportionate share of testicular cancer diagnoses in the Lanarkshire and Forth Valley in the same areas, is likely sentinel for reproductive health. Conflating these reproductive health signifiers however is exercised with caution given the multiplicity of potential factors and exposures involved. Notwithstanding this however, is that definitive proof of endocrine disruption for humans is almost impossible to establish, and public policy decisions must be reached on the basis of ‘the balance of probabilities’, by adopting the ‘Precautionary Principle’ (Howard 2001). Sharpe (2010), in reviewing environmental and health-related determinants of spermatogenesis both in its foundations at the peri-natal stage, and in adults, illustrates the need for reproductive biologists to investigate multiple effects. This population health study replicates such an approach, whereby the non-invasive, universal measure of secondary sex ratio may serve as an instructive reproductive health marker through inter-disciplinary inquiry.

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Appendices

APPENDIX A – PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	

Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**

APPENDIX B: Endocrine Disrupters listed on SEPA Scottish Pollutant Release Inventory

Common Name	**International (CAS)**	**Other Names**
2,4-DICHLOROPHENNOXYACETIC ACID (2,4-D) – ESTER AND NON-ESTER	94-75-7	Amoxane, Cloroxane, 2,4-D
2-ETHOXYETHANOL	110-80-5	Cellosolve, Poly-solv EE, Ethylene glycol monoethyl ether
ALACHLOR	15792-60-8	
ALDRIN	309-00-2	Octalene, Aldrec, Aldrex, Aldrite
AMITROLE	61-82-5	Weedazol, Hoechst Orchard Herbicide, 1H-1,2,4-Triazol-3-amine, 3-amino-1,2,4-triazole
AMMONIA	7664-41-7	ammonia gas, aqueous ammonia or ammonium hydroxide (when dissolved in water), NH3
ANILINE	62-53-3	Aminobenzene, Benzeneamine
ANTRACENE	07/12/20	Anthracin, paranaphthalene, Gruenoel
ARSENIC	7440-38-2	Compounds of arsenic
ASBESTOS	1332-21-4	Several varieties of asbestos exist- crocidolite, actinolite,anthophyllite, chrysotile, grunerite, tremolite, cristobalite.
ATRAZINE	1912-24-9	Residox, Primatol A, 6-Chloro-N-ethyl-N-isopropyl-1,3,5-triazinediyl-2,4-diamine
BENZENE	71-43-2	
BENZENE, TOLUENE, ETHYLBENZENE,		

XYLENE (BTEX)		
Common Name	**International (CAS)**	**Other Names**
BENZO(A) PYRENE FLUROANTHENE	50-32-8	benzo(d,e,f)chrysene, 3,4-benzopyrene, BaP
BENZO(B) FLUROANTHENE	205-99-2	
BENZO(K) FLUROANTHENE	207-08-9	
BIPHENYL	92-52-4	Lemonene, Xenene, Diphenyl,1,1'-biphenyl, Bibenzene
BISPHENOL-A	80-05-7	Diphenyol propane, 4,4'-(1-Methylidene)bisphenol, BPA
BROMINATED DIPHENYLETHERS		PBDEs
CADMIUM	7440-43-9	cadmium and compounds of cadmium
CARBON DISULPHIDE	75-15-0	Carbon bisulphide, Sulphocarbonic anhydride
CARBON MONOXIDE	630-08-8	coal gas, carbon(ic) oxide, flue or exhaust gas, CO
CARBON TETRACHLORIDE	56-23-5	
CHLORDANE	57-74-9	dichlorochlordene; chlordan; Corodane; Octachlor; Belt; Chlortox; Gold Crest; Intox; Synklor; Aspon, Chloriandrin, Chlorkil, Corodan, Dowchlor, Termi-Ded;1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene; 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydromethanoindan; octachlorro-4,7-methanotetrahydroindan dichlorochlordene
CHLORINE	7782-50-5	brine, saline (when in solution), chloride, chloride ion, hydrogen

		chloride
Common Name	**International (CAS)**	**Other Names**
CHLOROFORM	67-66-3	
CHLORPYRIFOS	2921-88-2	Dursban, Lorsban, "Destroyer", "Strike", O,O-diethyl-(3,5,6-trichloro-2-pyridinyl)phosphoroioate
CHRONIUM	7440-47-3	compounds of chronium
COPPER	7440-50-8	compounds of copper
CYANIDES		Total CN
CYPERMETHRIN	52315-07-8	Ammo, Avicade, Barricade
DELTAMETHRIN	52918-63-5	
DIAZINON	333-41-5	Basudin, Knox out, Dethlac, Murphy Root Guard, O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
DIBUTYLPHTHALATE	84-74-2	1,2-Benzenecarboxylicacide, dibutyl ester, DBP
DICHLORODIPHENYLTRICHLOROETHANE	50-29-3	Agritan, Azotox, Gyron, Dichloro-diphenyl-trichloro-ethane
DICHLOROVOS	62-73-7	DDVP,Vapona, Cyanophos, 2,2-Dichlorovinyl dimethy phosphate
DIELDRIN	60-57-1	Alvit, Dieldrite, Illoxol
DIMETHOATE	60-51-5	Fosfamid, Cygon
DIMETHYL FORMAMIDE	68-12-2	N-Formyldimethylamine, DMF
DINOSEB	88-85-7	DNBP, Haulmex, Chafer Desicoil, 2-(1-methylpropyl)-4,6-dinitrophenol

Common Name	**International (CAS)**	**Other Names**
DIOXINS AND FURANS – as ITEQ		polychlorinated dibenzofurans (PCDFs), polychlorinated-p-dioxins (PCDDs)
DIOXINS AND FURANS – as WHO TEQ		polychlorinated dibenzofurans (PCDFs), polychlorinated-p-dioxins (PCDDs)
DIURON	330-54-1	Direx, Karmex, Dichlorfendim, 3-(3,4-Dchlorophenyl)-1,1-dimethylurea
ENDOSULFAN	115-29-7	Thiodan, Endox, Malix
ENDRIN	72-20-8	Isodrin epoxide, Endrex, Hexadrin, Mendrin
EPICHLOROHYDRIN	106-89-8	ECH, 1-Chloro-2,3-epoxypropane
FENITROTHION	122-14-5	SMT, Methylnitrophos, Dicofen, Fentro
HEPTACHLOR	76-44-8	
HEXACHLOROBENZENE	118-74-1	"bunt cure"
HEXACHLOROBUTADEINE	87-68-3	C-6, Dolenpur
HYDROGEN CYANIDE	74-90-8	
IODINE		
LEAD	7439-92-1	compounds of lead
LINDANE	58-89-9	
LINURON	330-55-2	Afalon, Lorox, Linurex
MALATHION	121-75-5	Phosphothion, Sumitox, Calmathion
MANGANESE	7439-96-5	Mangnacat, Mangan, Tronamang, Cutaval
MERCURY	7439-97-6	compounds of mercury

METHANOL	67-56-1	
Common Name	**International (CAS)**	**Other Names**
METHYL BROMIDE	74-83-9	Curafume, Haltox, Terabol, Zytox, Bromomethane
MIREX	2385-85-5	
? NAPTHALENE	91-20-3	Camphor tar, Mothballs, Mighty 150
NITROGEN OXIDES		oxides of nitrogen
NONYLPHENOL ETHOXLATES		Nonoxynol, Antarox, Makon, NPEs, NPEOs
NONYLPHENOLS		NPs
OCTYLPHENOL ETHOXLATES		Igepal, Triton X-100, OPEs
OCTYLPHENOLS		OPs
PENTACHLOROPHENOL	87-86-5	
PERMETHRIN	52645-53-1	Permetrina, Ambush, Pounce, Perto
PHENOLS	108-95-2	phenolic compounds
POLYCHLORINATED BIPHENYLS (PCBs) – as WHO TEQ		Arochlor, Pyranol, Clophen, PCBs
POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)		benzo(a)pyrene, benzo(b)fluoranthrene, benzo(k)fluranthrene, indo(1,2,3-cd)pyrene., PAHs
SELENIUM	7782-49-2	
SIMAZINE	122-34-9	Gestatop, Chemicrop
STYRENE	100-42-5	Vinylbenzene, Ethenylbenzene, Styropor, Cinnamene
TETRABROMO- BISPHENOL A	79-94-7	Derakane, Bromdian, Tetrabrom, TBBPA
TOXAPHENE	8001-35-2	
TRIBUTYLIN COMPOUNDS	56573-85-4	TBTs

Common Name	International (CAS)	Other Names
TRIFLURALIN	1582-09-8	Digermin, Trigard, Treflan
VINYL ACETATE	108-05-4	
ZINC	7440-66-6	compounds of zinc

The following documents were cross-referenced:

IEH (2005) *Chemicals purported to be Endocrine Disrupters: A Compilation of Published Lists* (Web Report W20), Leicester, UK, MRC Institute for Environment and Health, available at http://www.le.ac.uk/ieh/

SEPA (2010) *Scottish Pollutant Release Inventory: Pollutant Fact Sheet*, Stirling, U.K., Scottish Environment Protection Authority, available at http://apps.sepa.org.uk/spripa/Pages/SubstanceSearc

APPENDIX C: Scottish Environment Protection Agency: Conditions on using our information

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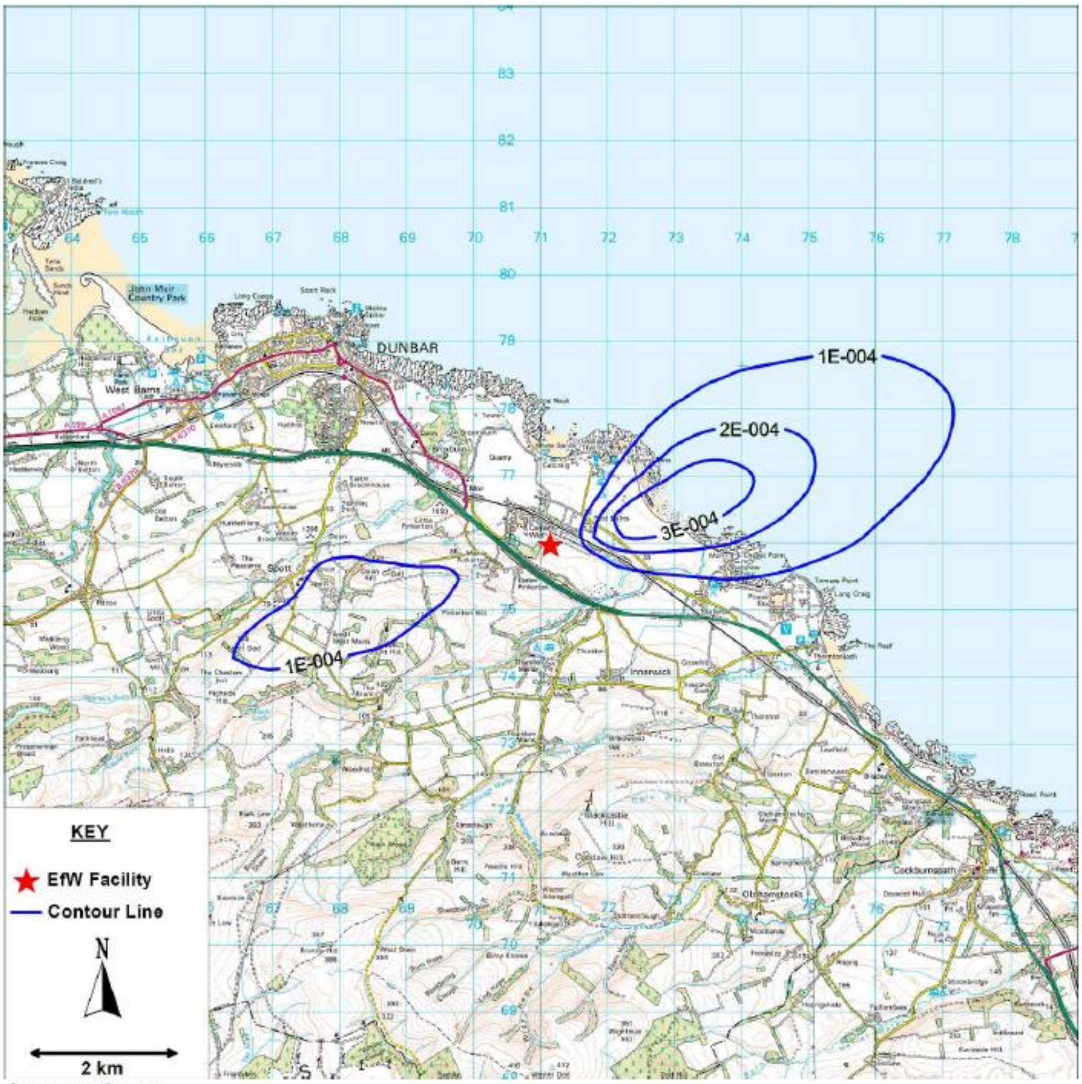
APPENDIX D: Contour Plot of Predicted Mean Contributions at Dunbar EfW

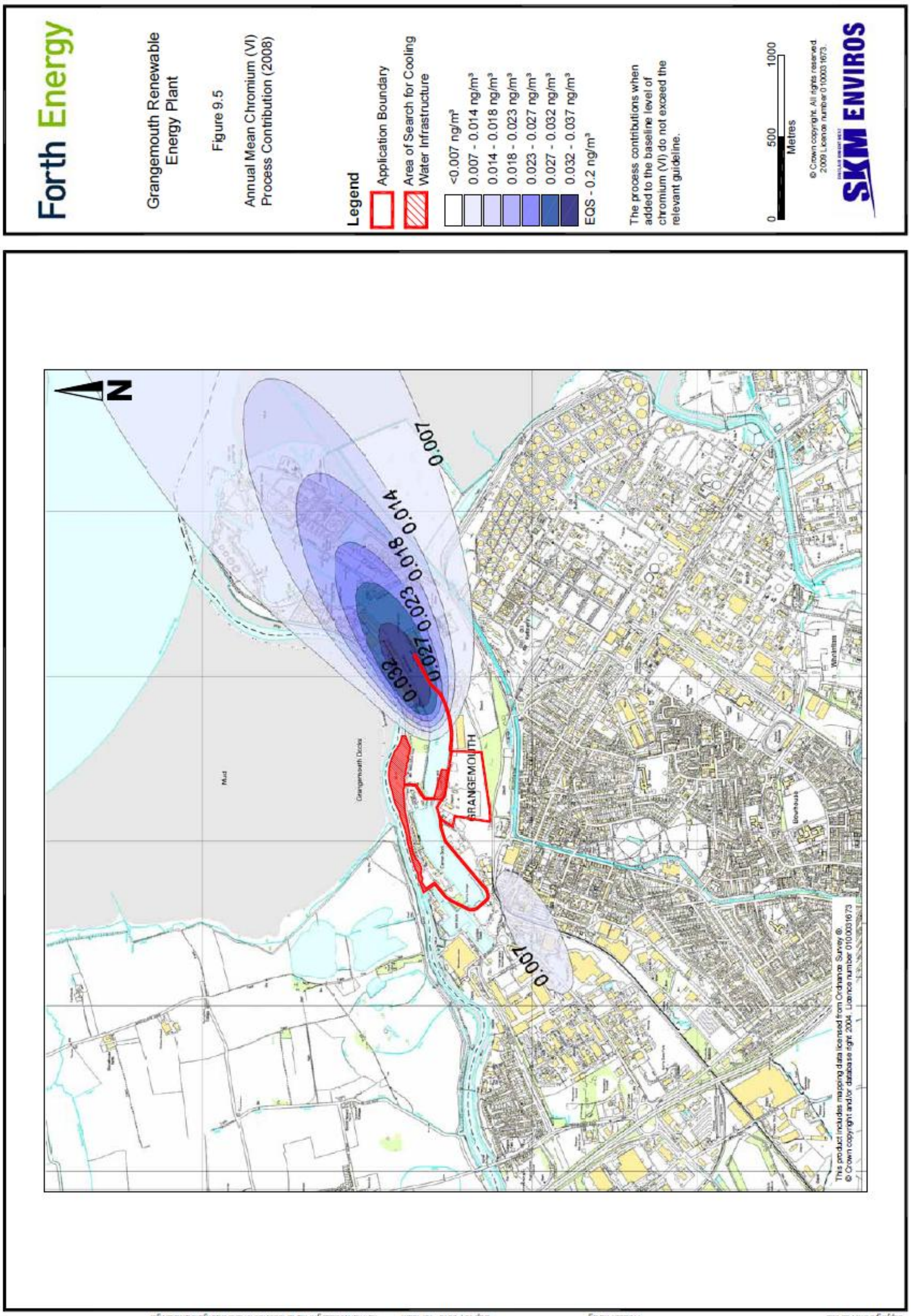
Figure 1.13: Contour Plot of Scenario 2 Predicted Annual Mean Cadmium Contributions: (µg.m^{-3})



Assumptions:

- Concentrations in µg.m^{-3}
- Proposed EfW operating at WID long-term limits
- 2001 meteorological year (worst case)
- Derived from AERMOD modelling (worst case)

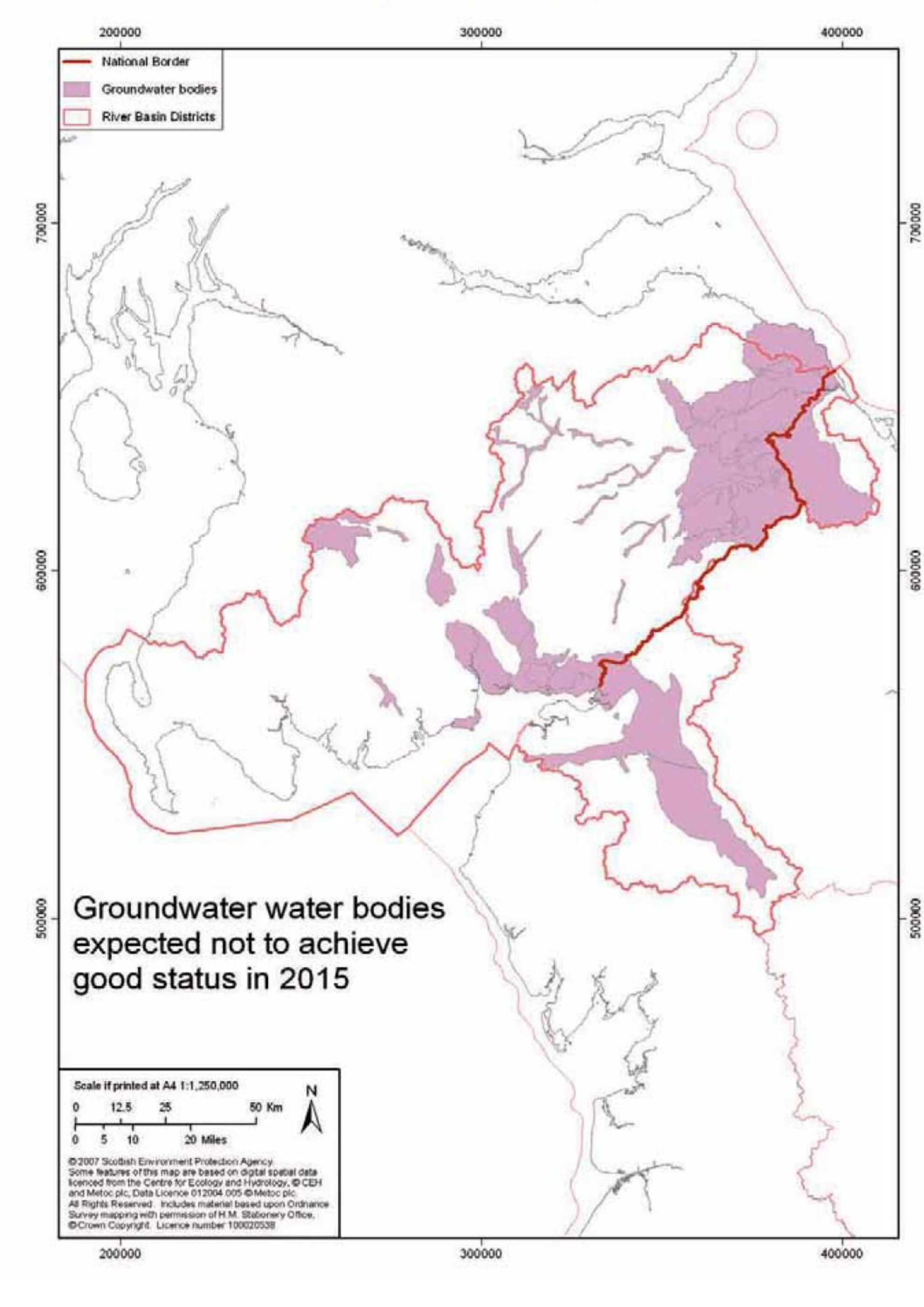
APPENDIX E: Annual Mean Chromium Process Contribution for Grangemouth EfW



Forth Energy (2012) *Biomass Project Update: Grangemouth Renewable Energy Plant:*

APPENDIX F: Solway Tweed river basin district: Groundwater water bodies expected NOT to achieve good status in 2015

Map D2: Groundwater water bodies expected not to achieve good status by 2015



Scottish Environmental Protection Authority, Environment Agency (2007) *An interim overview of the significant water management issues in the Solway Tweed river basin district*

APPENDIX G – Large Sample Significance Test Syntax

```
DEFINE pz_lt_zi (!POSITIONAL !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
compute Zi_Var = !1 .
COMPUTE PROB = CDFNORM(Zi_Var).
execute.
MATRIX.
GET PROB_VAR /VARIABLES = PROB.
GET Zi_Var /VARIABLES = Zi_Var.
COMPUTE Prob = PROB_VAR(1).
COMPUTE zi = Zi_Var(1).
COMPUTE ANSWER = {zi, PROB}ro.
PRINT ANSWER / FORMAT "F10.5" /Title = " Prob(z < zi) for a given zi " /
CLABELS = zi, Prob.
END MATRIX.
!ENDDEFINE.
```

```
DEFINE pz_gt_zi (!POSITIONAL !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
compute Zi_Var = !1 .
COMPUTE PROB = 1 - CDFNORM(Zi_Var).
execute.
MATRIX.
GET PROB_VAR /VARIABLES = PROB.
GET Zi_Var /VARIABLES = Zi_Var.
COMPUTE Prob = PROB_VAR(1).
COMPUTE zi = Zi_Var(1).
COMPUTE ANSWER = {zi, PROB}.
PRINT ANSWER / FORMAT "F10.5" /Title = " Prob(z > zi) for a given zi " /
CLABELS = zi, Prob.
END MATRIX.
!ENDDEFINE.
```

```
DEFINE pz_lg_zi (zil = !ENCLOSE('(',')')  / ziu =  !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
compute ZiL_Var = !zil .
compute ZiU_Var = !ziu .
execute.
COMPUTE PROBL = CDFNORM(ZiL_Var).
COMPUTE PROBU = 1 - CDFNORM(ZiU_Var).
COMPUTE PROBLG = PROBL + PROBU.
execute.
MATRIX.
GET PROB_VAR /VARIABLES = PROBLG.
```

```
GET ZiL_Var /VARIABLES = ZiL_Var.
GET ZiU_Var /VARIABLES = ZiU_Var.
COMPUTE Prob = PROB_VAR(1).
COMPUTE ziL = ZiL_Var(1).
COMPUTE ziU = ZiU_Var(1).
COMPUTE ANSWER = {ziL, ziU, PROB}.
PRINT ANSWER / FORMAT "F10.5" /Title = " Prob((z < ziL) OR (z > ziU)) for a
given zi " / CLABELS = ziL, ziU, Prob.
END MATRIX.
!ENDDEFINE.



DEFINE pz_gl_zi (zil = !ENCLOSE ('(',')')  / ziu = !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
compute ZiL_Var = !zil .
compute ZiU_Var = !ziu .
execute.
COMPUTE PROBL = CDFNORM(ZiL_Var).
COMPUTE PROBU = 1 - CDFNORM(ZiU_Var).
COMPUTE PROBLG = 1 - (PROBL + PROBU).
execute.
MATRIX.
GET PROB_VAR /VARIABLES = PROBLG.
GET ZiL_Var /VARIABLES = ZiL_Var.
GET ZiU_Var /VARIABLES = ZiU_Var.
COMPUTE Prob = PROB_VAR(1).
COMPUTE ziL = ZiL_Var(1).
COMPUTE ziU = ZiU_Var(1).
COMPUTE ANSWER = {ziL, ziU, PROB}.
PRINT ANSWER / FORMAT "F10.5" /Title = " Prob(ziL < z < ziU) for a given zi " /
CLABELS = ziL, ziU, Prob.
END MATRIX.
!ENDDEFINE.


DEFINE zi_lt_zp (p = !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
COMPUTE Zi = PROBIT(!p).
EXECUTE.
MATRIX.
GET Zi_VAR /VARIABLES = Zi.
COMPUTE Zi = Zi_VAR(1).
COMPUTE PROB= {!p}.     /*Enter the given probability into the curly brackets*/
COMPUTE ANSWER = {Zi, PROB}.
PRINT ANSWER / FORMAT "F10.5" /Title = " Value of zi such that Prob(z < zi) =
PROB when PROB is given" / CLABELS = zi, PROB.
END MATRIX.
!END DEFINE.
```

```
DEFINE zi_gt_zp (p = !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
COMPUTE Zi = PROBIT(1-!p).
EXECUTE.
MATRIX.
GET Zi_VAR /VARIABLES = Zi.
COMPUTE Zi = Zi_VAR(1).
COMPUTE PROB= {!p}.     /*Enter the given probability into the curly brackets*/
COMPUTE ANSWER = {Zi, PROB}.
PRINT ANSWER / FORMAT "F10.5" /Title = " Value of zi such that Prob(z > zi) =
PROB when PROB is given" / CLABELS = zi, PROB.
END MATRIX.
!END DEFINE.


DEFINE zi_gl_zp (p = !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
COMPUTE PROB = !p.
COMPUTE PROBLG = 1 - !p.
COMPUTE PROBL = PROBLG / 2.
COMPUTE ZiL_Var = PROBIT(PROBL).
COMPUTE ZiU_Var = -1 * ZiL_Var .
execute.
MATRIX.
GET PROB_VAR /VARIABLES = PROB.
GET ZiL_Var /VARIABLES = ZiL_Var.
GET ZiU_Var /VARIABLES = ZiU_Var.
COMPUTE Prob = PROB_VAR(1).
COMPUTE ziL = ZiL_Var(1).
COMPUTE ziU = ZiU_Var(1).
COMPUTE ANSWER = {ziL, ziU, PROB}.
PRINT ANSWER / FORMAT "F10.5" /Title = " Value of zi such that Prob(-zi < z < zi)
= PROB, when PROB is given  " / CLABELS = ziL, ziU, Prob.
END MATRIX.
!ENDDEFINE.



DEFINE H_L2P (n1 = !ENCLOSE('(',')')  / n2 = !ENCLOSE('(',')')  / x1 =
!ENCLOSE('(',')')  / x2 = !ENCLOSE('(',')')).
GET FILE='C:\QUANTS\one.sav'.
MATRIX.
COMPUTE n1 = {!n1}.  /* Enter the first sample size here */
COMPUTE n2 = {!n2}.  /* Enter the second sample size here */
COMPUTE x1 = {!x1}.  /* Enter the number of "successes" or particular outcomes for
sample 1 here */
COMPUTE x2 = {!x2}. /* Enter the number of "successes" or particular outcomes for
sample 2 here */
```

```
COMPUTE p1 = x1/n1.
COMPUTE p2 = x2/n2.
COMPUTE phat = (x1 + x2) / (n1 + n2).
COMPUTE SE_phat = SQRT(phat * (1 - phat) * ((1/n1) + (1/n2))).
COMPUTE z = (p1 - p2) /SE_phat.
COMPUTE SIGz_2TL = 2 * (1 - CDFNORM(ABS(z))).
COMPUTE SIGz_LTL = CDFNORM(Z).
COMPUTE SIGz_UTL = 1 - CDFNORM(Z).
COMPUTE ANSWER = {p1, p2, SE_phat, z, SIGz_2TL, SIGz_LTL, SIGz_UTL}.
PRINT ANSWER / FORMAT "F10.5" /Title = "Large sample sig. test for two
proportions" / CLABELS = p1, p2, SE, z, SIGz_2TL, SIGz_LTL, SIGz_UTL.
END MATRIX.
!ENDDEFINE.
```

```
H_L2P    n1=(  [n figure for 1st region]  )    n2=( [n figure for 2nd region] )  x1=( [n
figure for men in 1st region] )  x2=(n figre for men in 2nd region) .
```

e.g.

```
H_L2P    n1=(46)  n2=(546307) x1=(20) x2=(279941)
```

APPENDIX H: Andrology Australia Booklet

Source: https://www.andrologyaustralia.org/wp-content/uploads/BL-Spermbooklet_final.pdf (full document can be downloaded at this website)