A QUANTITATIVE RISK ASSESSMENT OF EXPOSURE TO NITRATES IN DRINKING WATER AND THYROID DISORDERS IN EAST ANGLIA, UNITED KINGDOM

GEORGE NNAMDI ONUOHA

A thesis submitted for the degree of Doctor of Philosophy in PUBLIC HEALTH

School of Health and Human Sciences

University of Essex

Date of Submission: June 2018

TABLE OF CONTENTS

Abstract……..6
List of Tables…….....7
List of Figures……..8
Acknowledgement…….…..9
Dedication ……10
Glossary of Terms……..……11

CHAPTER ONE…….....13

1.0 Introduction……..….13
1.1 Background to the study…….....16
1.2 Rationale for the study: Nitrate and human health-current state of Knowledge……...21
1.3 Outline of the thesis…….…..24

CHAPTER TWO: NITRATES AND DRINKING WATER CONTAMINATION: OVERVIEW……....25

2.1 Introduction…….…..25
2.2 Legislative framework for managing water quality……...28
2.3 Nitrate concentration in drinking water sources in the UK……...…31
2.4 Trend in nitrate concentrations in the UK…….....34
2.5 Routes of exposure…….....38
2.6 Toxicokinetics……39
2.6.1 Absorption and Distribution……..39
2.6.2 Metabolism and Biotransformation……..…….41
2.6.3 Endogenous nitrate synthesis…….....41
2.6.4 Excretion……42
2.7 Toxicodynamics…….....43
2.7.1 Mode of action of Nitrates on the thyroid gland……44
2.8 Summary……48

CHAPTER THREE: LITERATURE REVIEW…….……...50

3.1 Introduction……50
3.2 Thyroid disorders: Review of animal studies……55
3.3 Thyroid disorders: Review of epidemiological Studies…….63
3.4 Discussion……..84
3.4.1 Meta – Analysis…….85
3.4.2 Weight of Evidence Assessment……...95
3.4.3 Gaps in the Literature……..101
3.5 Conclusion……...102

CHAPTER FOUR: RESEARCH METHODOLOGY…….……104

4.1 Aims and Objectives…….…...104
4.2 Quantitative Risk Assessment…….....106
4.3 Discussion……109
4.3.1 Evidence - Based Public Health….....111
4.3.2 Public Health Strategies in England…….……...113
4.4 Description of the study Area……..115
4.5 Sources of Data: (Data Collection)……..117
4.5.1 Water Companies (public water)…….117
4.5.2 Local Authority (private water supply)……...118
4.5.3 Data on tap-water intake..…….....119
4.6 Ethical Approval……..120
4.7 Summary……..121

CHAPTER FIVE: DOSE – RESPONSE ASSESSMENT…….122

5.1 Introduction…….122
5.2 Dose – Response Analysis……...124
5.3 Low – dose extrapolation…….125
5.4 Discussion……129
5.5 Conclusion……..…….134

CHAPTER SIX: EXPOSURE ASSESSMENT……..136

6.0 Exposure Assessment……. 136
6.1 Data Analysis ……..136
6.1.1 Calculating the amount of water intake…….…..138
6.2 Result……...139
6.3 Discussion……147
6.3.1 Risk assessment based legislation on private water supply…….152
6.4 Conclusion……...156

CHAPTER SEVEN: RISK CHARACTERISATION……158

7.1 Introduction…….158
7.2 Integrating dose-response and exposure assessments……161
7.2.1 Dose – response assessment…….....161
7.2.2 Exposure assessment…….....161
7.3 Result……..165
7.4 Discussion……172
7.4.1 Public health implications……177
7.5 Uncertainty and limitations of the study……..181
7.6 Conclusion ……..186

CHAPTER EIGHT: DISCUSSION; CONCLUSION AND RECOMMENDATIONS FOR FURTHER STUDIES……190

8.1 Summary of Findings……...191

8.2 Discussion……197
8.2.1 Implications on Public Health Policy…….....202
8.2.2 Reduction of nitrate concentration in drinking water: cost - benefit analysis…….206
8.3 Conclusion…….....211
8.4 Recommendations……214
8.5 Further Research…….....215

REFERENCES……..217

APPENDICES……...285

Appendix 1: Database literature search terms……285
Appendix 2: Funnel Plot for hyperthyroidism, hypothyroidism and goitre…….286
Appendix 3: Letter to water companies requesting nitrate data in public water…….....289
Appendix4: Letter to local authorities requesting nitrate data in private water supplies……..290
Appendix 5: Average concentration of nitrates in private and public water supplies and the population served……..292
Appendix 6: Questionnaire to residents of Suffolk Coastal District Council requesting information of water intake…….....295
Appendix 7: Ethical approval……298
Appendix 8: Health Protection Agency (Public health England) Advice Note……299
Appendix 9: Table on excess risk of thyroid cancer by age in East Anglia……..300

ABSTRACT

Review of animal and epidemiological studies suggest that exposure to nitrates in drinking water is associated with thyroid disorders, including mild - to - moderate iodine deficiency; hyperthyroidism; hypothyroidism; thyroid hypertrophy (goitre) and thyroid cancer. However, the weight of evidence following a meta – analysis is strongest for goitre; weak for subclinical hypothyroidism and weakest for clinical hypothyroidism and hyperthyroidism (clinical and subclinical). The effect estimate for goitre is, OR = 3.13 (95%Cl: 2.35-4.16); I^2 = 24.9%, p = 0.28. Although causality was not firmly established between nitrates in drinking water and goitre, the risk assessment framework was used to estimate lifetime excess risk of thyroid cancer in East Anglia given widespread nitrate contamination of drinking water sources in the region. Thyroid cancer was used as a proxy for goitre given that malignancy can result from goitre which is usually benign and there is no register for goitre and/or benign thyroid tumours in the UK.

Risk estimates suggests that 20 cases or 13 per cent of the 154 thyroid cancer cases calculated in a population of 2,849,918 in East Anglia in 2014, can be attributable to nitrates in drinking water and this would have been eliminated from the population if there was no nitrates in drinking water. The lifetime excess risk of thyroid cancer at nitrate levels below and equal to the drinking water standard of 50mg/l, is 0.02 – 0.28. This is above the range ($1x10^{-6}$ to $1x10^{-5}$) considered negligible and suggests that the current drinking water standard for nitrates, originally set to protect against infantile methaemoglobinemia is unlikely to protect against thyroid cancer and warrants a review. The review should include a consideration of lowering the drinking water standard; reduction of nitrates in drinking water sources and/-or introducing iodine prophylaxis in the UK given that the effect of nitrates on the thyroid gland is dependent on the amount of dietary iodine intake. Although there were a lot of uncertainties and assumptions in the risk assessment process, the recommendation is based on the precautionary principle.

LIST OF TABLES

Table 1: Summary of animal studies on thyroid disorders……..56

Table 2: Summary of epidemiological studies on thyroid disorders.....64

Table 3: Nitrate levels in public and private water supplies and the approximate population served in East Anglia in 2001-2010 (Appendix 5)……..……..292

Table 4: Characteristics of respondents to questionnaire on water intake……139

Table 5: Contact rate (CR) calculation - amount of water intake by age group……...141

Table 6: Parameters used in calculating chronic daily (CDI) water intake…….143

Table 7: Chronic daily intake of nitrates in drinking water……..145

Table 8: Number of private water supplies per region in England……..148

Table 9: Risk estimates of thyroid cancer in East Anglia…….167

Table 10: Number of Private Water Supplies and thyroid cancer cases by region in England……...177

Table 11: Overview of uncertainty…….. ……..…….184

LIST OF FIGURES

Figure 1: Map showing the distribution of Private Water Supplies by region in England……...18

Figure 2: The nitrogen cycle …….…….....26

Figure 3: Map showing Nitrate Vulnerable Zones in East of England……30

Figure 4: Relative contributions of surface water, groundwater and other sources of drinking water in EU14…….....33

Figure 5: Percentage of rivers with high nitrate concentrations in England & Wales 1995-2005…….....35

Figure 6 Trend in groundwater nitrate concentrations in England and wales 1980 – 2001……..36

Figure 7 PRISMA Flow Diagram …….....54

Figure 8 Forest Plot of clinical hyperthyroidism (random effect model)…….. ….....88

Figure 9 Forest Plot for subclinical hyperthyroidism (random effect model)……89

Figure10 Forest Plot for clinical hypothyroidism (random effect models)…….90

Figure 11 Forest plot for subclinical hypothyroidism (random effect model)…….....91

Figure12 Forest plot for subclinical hypothyroidism (sensitivity analysis)…….92

Figure13 Forest Plot for goiter (random effect models)……..93

Figure 14 Forest Plot for goiter (sensitivity analysis)…….....94

Figure 15: Map of East Anglia……..115

Figure 16: Map of nitrate vulnerable Zones in England……..…….116

Figure 17 Dose – response relationship between nitrates and goiter……...124

Figure 18 Map of England showing areas where more than 1% of population do not have access to public supply..……..149

ACKNOWLEDGEMENT

I am very grateful to God for the strength and resources to complete this study. I am grateful to my supervisors, Professor Ian Colbeck and Mr Allan Hildon for their support and guidance in completing this thesis. I also acknowledge the contributions and guidance of my previous supervisors, Dr Camilo Chinamasa and Dr Valarie Thurtle in the early stages of this study.

Thanks to my director, Mr Phil Gore and former manager, Mr Tim Davidson for giving me time off work to undertaken this study and for allowing me to use nitrate data from private water supplies in Suffolk Coastal DC in this study. I am equally grateful to the Environmental Health Managers of Local Authorities in East Anglia for approving my request for data on nitrate concentrations in Private Water Supplies in their respective areas and for allowing me to use such data in this study.

I also want to thank the Water Quality Manager of Essex & Suffolk Water; Cambridge Water; Tendering Hundred and Anglian Water Companies for providing nitrate concentration data in Public supply and the population served by their respective companies. I am grateful to Bob Markall; Phil Hitchens and Claire Moody of Anglian Water for their insight on water supply zones and water 'blending' and for providing data and maps with population served per water supply zone. I thank most sincerely Professor Graham Horgan and Dr Claus Meyer of the Biostatics Centre of the University of Aberdeen for their training and advice on meta-analysis.

A special thanks to my wife and children for their understanding and support without which this study would have be more difficult.

DEDICATION

Whatever you have learned or received or heard from me, or seen in me--put it into practice.

And the God of peace will be with you.

Philippians 4 v 9 (NIV)

I dedicate this thesis to my wife and children.

GLOSSARY OF TERMS

AF	Attributable Fraction
ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centre for Disease Control
CDI	Chronic Daily Intake
CDSC	Communicable Disease Surveillance Centre
CNS	Central Nervous System
DEFRA	Department of Environment, Food and Rural Affairs
DoH	Department of Health
DWI	Drinking Water Inspectorate
EBD	Environmental Burden of Disease
EBPH	Evidence Based Public Health
EC	European Commission
EEA	European Environment Agency
EU	European Union
EWG	Environmental Working Group
Hb	Haemoglobin
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
IARC	International Agency for Research on Cancer
JSNA	Joint Strategic Needs Assessment
JHWS	Joint Health and Wellbeing Strategies
MethHb	Methaemoglobin
NAS	National Academy of Science
NHS	National Health Service
NIS	Sodium Iodide Symporter
NO	Nitric Oxide
NOC	N-nitroso-compounds
NRC	National Research Council
NVZ	Nitrate Vulnerable Zone

OR	Odd Ratio
PAF	Population Attributable Fraction
PWS	Private Water Supplies
RR	Relative Risk
SGV	Soil Guideline Value
UNICEF	United Nations International Children Education Fund
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
WoE	Weight of Evidence

CHAPTER ONE

1.0 INTRODUCTION

Water is regarded as the most important natural resource in the world and is essential for the sustenance of life on earth since without it, no life will exist (Tebbutt, 1998). Water has a wider influence on human health and well-being as both the quantity and quality of water available for drinking and sanitation are important in determining the health of individuals and whole communities (World Health Organization (WHO), 2011a). Access to adequate, safe and reliable water is therefore a major prerequisite for a healthy life (WHO, 2011b). Although water is essential for the existence of life, it also plays a role in disease transmission and prevention (WHO, 2011a). Failure to achieve high quality drinking water exposes the population (particularly young children, the elderly, the sick and those living in poor sanitary conditions) to the risk of water-related diseases (WHO, 2011a). Poor microbial water quality has been implicated in the spread of some important infectious and parasitic diseases such as cholera; typhoid; dysentery; diarrhoea; hepatitis; giardiasis; guinea worm and schistosomiasis (WHO, 2011a). For example, diarrhoeal disease due to poor microbial water quality is a major public health issue worldwide and is reported to account for approximately 4.1 per cent of the total global burden of disease, resulting in about 1.8 million deaths annually (Pruss-Ustun & Corvalan, 2006; WHO, 2011c).

The main objective in water quality control works is for public health protection from the incidence of waterborne diseases (Tebbutt, 1998) by supplying ‘wholesome’ water that meets WHO microbiological and chemical standards (Drinking Water Inspectorate (DWI), 2011). Although microbial contamination of drinking water is widespread and exposure to such water

and its attendant health consequences are well reported (Ritter et al, 2002), chemical contamination of drinking water sources is also a growing concern in many regions of the world (Ritter et al, 2002; WHO, 2011b). Like microbial contaminated water, exposure to chemical contaminated water can also adversely affect human health, although in general such effects tend to be chronic rather than acute unless a specific pollution event has occurred (WHO, 2011a). Chemical contaminants usually found in drinking water sources include nitrates, arsenic, mercury, fluoride etc. Whilst some of these chemicals are naturally occurring (e.g. nitrates, fluoride, arsenic), some can inadvertently be introduced into drinking water sources (e.g. nitrates, pesticides) by anthropogenic activities (e.g. agricultural activities) and are reported to pose significant health risks to human health (WHO, 2011a).

In the United Kingdom (UK), although waterborne diseases due to microbial contamination of drinking water sources are no longer widespread in comparison to developing countries due to improved drinking water quality, chemical contamination of drinking water sources remains a public health challenge (DWI, 2011), and can place excessive burden on the health of the population and the health service (WHO, 2011a). Nitrates have been implicated in the deterioration of drinking water quality, especially groundwater in many parts of the UK in the last four to five decades (Environment Agency, 2011). In many parts of the country, nitrate concentrations in excess of the WHO drinking water standard of 50mg/l have been reported in drinking water sources, particularly private wells and boreholes (Private Water Supplies (PWS) (Environment Agency, 2011).

Exposure to nitrates in drinking water has long been associated with infantile methaemoglobinemia (blue-baby syndrome) (Comly 1945; 1987) and the current drinking water standard of 50mg/l was set by the WHO to protect infants from this disease (WHO 1958;

Environmental Working Group (EWG) 1996). No other health condition was considered in setting this standard or guideline value (EWG, 1996; Ward et al, 2005). Recently, the International Agency for Research on Cancer (IARC) classified nitrate and/-or nitrite as a probable human carcinogen (Group 2A) on the basis of limited evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity in experimental animals (IARC, 2010). There have been reported cases of adverse reproductive outcomes, and diabetes as a result of nitrate exposure the drinking water (Ward et al, 1996; Weyer et al, 2001; DeRoos et al, 2003; Brender et al, 2004) (although no cause and effect relationship was firmly established) but the appropriateness of the current drinking water standard for nitrates in protecting against these potential health outcomes has not been previously evaluated. Experimental animal studies also suggest that exposure to nitrates can inhibit iodine uptake by the thyroid gland resulting in thyroid disorders (Bloomfield et al, 1961; 1962; Jahries et al, 1986), and the effect may not be different in humans (Ward et al, 2005).

This study therefore aims to evaluate the relationship between exposure to nitrates in drinking water and thyroid disorders and to quantify any risk to the population in East Anglia. East Anglia is one of the Nitrate Vulnerable Zones (NVZ) in the UK where drinking water sources are known to be vulnerable to nitrate pollution (Department for environment, Food & Rural Affairs (DEFRA), 2006; 2009). The study was conducted following the United States National Academy of Sciences (NAS) risk assessment framework (National Research Council (NRC), 1983; 2008). The four stages of the risk assessment process are: hazard identification; dose-response (effect) evaluation; exposure (source, pathway) assessment; and risk characterisation (NRC, 1983; 2008; Ritter et al, 2002). Each of the four stages of risk assessment addresses different questions that can be used to determine any anti-thyroid effect of nitrates; the concentration of nitrates in drinking water in the study area; the frequency and magnitude of exposure in the population; the

most vulnerable groups in the population and excess risk of thyroid disorders in the population (if any risk exists).

1.1 BACKGROUND TO THE STUDY

Access to satisfactory (adequate, safe) drinking water is essential for health, a basic human right, and a major component of public health protection policy (WHO, 2011b). Inadequate or poor drinking water quality is among the world's major causes of preventable morbidity and mortality (WHO, 2003a). Today, the vast majority of the population in the UK are served with high quality drinking water by statutory water undertakers (water companies). These public supplies which are abstracted from groundwater and surface water sources usually undergo physical treatment (e.g. reverse osmosis filtration) and chemical treatment (e.g. chlorination) with the aim of providing 'wholesome' water to the consumer that meets microbial and chemical standards as specified in the Water Supply (Water Quality) Regulations 2000 (amended in 2001). In England &Wales, the regulation of drinking water is by the Drinking Water Inspectorate (DWI) and the legislative and water quality control measures over public supplies have greatly reduced the incidence and or outbreak of waterborne diseases in the UK (DWI, 2011).

However, not everybody in the UK is served by public water as there is the population whose only source of drinking water is from private well or borehole (Private Water Supplies) (DWI, 2006; Environment Agency, 2006). A private water supply (PWS) is defined in Section 93 of the Water Industry Act 2003 as, "any supply of water provided otherwise than by a statutorily appointed water undertaker" (Water Act 2003). In other words, they are water supply which is not provided by water companies or licensees, but instead, they are the responsibility of the owners or users. The quality and safety of PWS is regulated by the PWS Regulation 2009

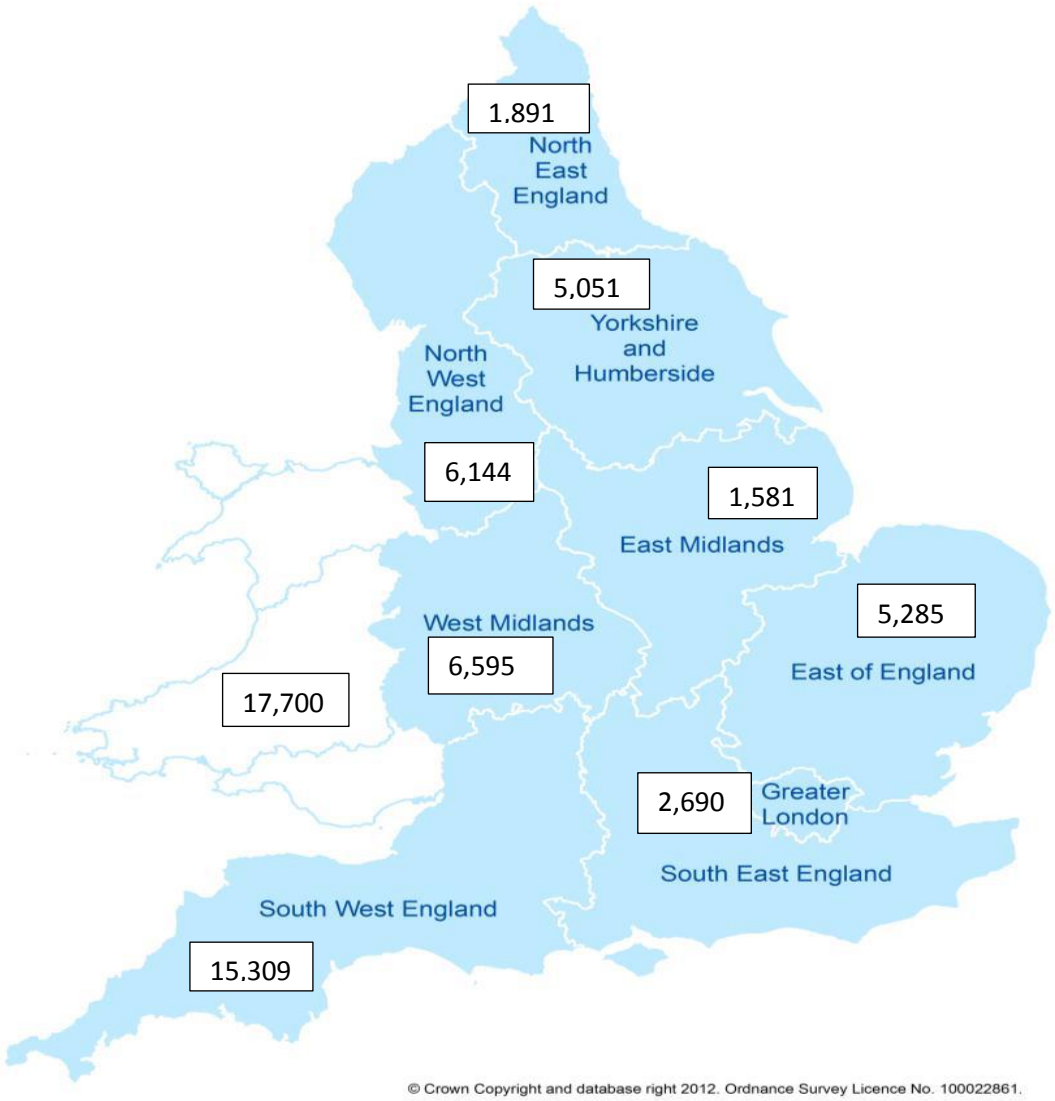
(previously PWS Regulations 1991) which implements the European Drinking Water Directive 98/83/EC. Although the standards and principles of regulation of PWS are the same for public water, it has been recognised that these supplies are more likely to be of poor quality and as a result have been linked to illness more than public supplies (DWI, 2006). PWS vary in their nature and range from shallow wells or boreholes serving individual houses, to deeper boreholes serving multiple dwellings, hotels, businesses, holiday accommodations, leisure facilities and tourist campsites. Whilst the majority of these private wells or boreholes are known to serve households mostly located in remote parts of the country and far from the nearest public water supply, some can be found in large towns and cities serving factories, hotels, business parks, educational centres, visitor attractions and health care centres (DWI, 2006).

According to DWI (2013), there are approximately 86,218 PWS in the UK serving a population of more than one million people. In England, DWI (2013) reported that there are about 44,546 PWS (51.7 per cent of UK total) serving a resident population of about 872,746 (1.6 per cent of England population) and a further 7.8 million people exposed to them when travelling through or taking a holiday in more rural areas of the country or when attending festivals, shows or other events served by a temporary supply of water. Figure 1 shows the distribution of PWS in England & Wales and suggest that the majority of these supplies are in the South West, North West, West Midlands, East of England (East Anglia), Yorkshire and Humberside (DWI, 2013). Generally, private wells are more susceptible to chemical and microbiological contamination because they are usually smaller; shallower; less well equipped; may not have any form of treatment (e.g. ultra-violet unit) and closer to sources of contamination when compared with public supply boreholes which are usually in deeper groundwater aquifers where contamination is less likely (US Geological Survey (USGS), 1996). Also PWS are subject to less strict regulation and surveillance than public supplies and therefore more likely to suffer water quality

failures (Natural Environment Research Council (NERC), 2002; Manassaram et al, 2006). Thus, the health of those consuming water from this type of water supply is potentially at risk from microbial and chemical contamination (Manassaram et al, 2006).

Figure 1

Map showing distribution of private water supplies by region in England (Source: DWI, 2013).



Whilst improvements in the quality of public water supplies have greatly reduced the incidence of waterborne diseases, microbial and chemical contamination of PWS remain the major cause

of waterborne disease in the UK (DWI, 2011). Although the quality of public water in England has improved over the years, the same cannot be said of PWS (DWI, 2014). For example whilst only about 0.03 per cent of public water failed to meet WHO microbial and chemical standards in 2013 (0.04 per cent in 2012), about 9.6 per cent of PWS in 2010 (7.5 per cent in 2013) failed to meet the WHO and European Union (EU) drinking water chemical standards (DWI, 2014).

According to the DWI (2013), 782 failures were recorded in water samples collected from PWS in England across 21 chemical parameters measured against WHO and EU drinking water standards in England in 2012. Whilst nitrate accounted for 58 per cent of such failures, lead accounted for 8 per cent arsenic; fluoride and copper each accounted for 9 per cent failures. In 2014, the main chemicals identified in failures to meet the drinking water standards were nitrate (56 per cent) and lead, (18 per cent). In the same year, 32 different pesticides were detected in PWS, of which 12 were approved for agriculture use in the UK (DWI, 2015). Like chemical contamination, microbial contamination also continue to pose a risk to water quality in PWS in England & Wales, with about 12.8 per cent of water samples containing Esherichia coli (*E.coli)* and 13.4 per cent containing *Enterococci* in 2014. The presence of these two micro-organisms in drinking water suggests that the water supply is contaminated with faecal matter and therefore contain disease pathogens (DWI, 2015). All these indicate the vulnerability of PWS to chemical and microbial contamination and highlight the need for public health protection from contaminants in these types of drinking water sources. Whilst more than 5 per cent of the resident population in East Anglia and parts of Devon have been reported to rely solely on PWS for their drinking water, DWI recommends that the safety of PWS should be an integral part of the affected Local Authority's Public Health Protection Strategy (DWI, 2014). Unlike public water, monitoring of PWS in England and Wales to ensure wholesomeness is the responsibility

of Local Authority Environmental Health Officers as prescribed in the Private Water Supply Regulations 2009 (DWI, 2011).

As part of the Environmental Health Team of Suffolk Coastal District Council, it is our responsibility to implement the PWS Regulation in order to ensure that water from such supply is 'wholesome' i.e. complies with the microbiological and chemical standards, equivalent to the Prescribed Concentrations or Values (PCVs) contained in the Water Supply (Water Quality) Regulations 2000. The PCVs contained in the Water Supply (Water Quality) Regulations 2000 are derived from Annex 1 of European Drinking Water Directive 98/83/EC and are based on WHO Guidelines for drinking water quality' (WHO, 2004b). The implementation of this legislation involves the collection of water samples from the water-taps of houses served by PWS and laboratory analysis for parameters such as coliform bacteria; turbidity; pH; heavy metals; nitrates; pesticides etc.

Concern has been expressed in recent times by the population in Suffolk Coastal District Council served by PWS about the quality of their drinking water and the potential health impact that may be associated with exposure to nitrates in their drinking water. These concerns stem from high nitrate concentrations recorded in these supplies by the District Council in implementing the PWS Regulation 1991 (now PWS Regulation 2009). In some of these supplies, nitrate concentrations of 460mg/l, well in excess of the drinking water standard of 50mg/l have been recorded. The high concentration of nitrates recorded in these supplies prompted my Team in 2005 to enquire from the Environmental Health Departments of other Local Authorities in East Anglia (Suffolk; Norfolk; Essex and Cambridgeshire) about the concentration of nitrates recorded in PWS in their respective areas. The result of this survey (unpublished) also showed high concentration of nitrates in PWS in these areas, indicating that nitrate contamination of

drinking water sources was widespread in East Anglia. High nitrate concentration in drinking water and its potential adverse health effects is a public concern and the current advice from the DWI and Public Health England to my team is to take a precautionary approach by taking enforcement action on PWS where nitrate concentrations greater than 100 mg/l are detected. Such enforcement action may require the installation of water filters to the water-taps of affected houses or service of Improvement Notices.

1.2 RATIONALE FOR THE STUDY: NITRATE AND HUMAN HEALTH - CURRENT STATE OF KNOWLEDGE

The only established human health effect associated with exposure to nitrate in drinking water is infantile methaemoglobinemia (blue-baby syndrome) (Comly 1945, 1987; WHO, 2006a). This condition is thought to result from bacterial reduction of ingested nitrate in the stomach to nitrite and then to nitric oxide or the acidification of nitrite to nitric oxide (Mcknight, 1999; Agency for Toxic Substances and Disease Registry (ATSDR), 2001; Bjorne, 2005). In the bloodstream, nitric oxide can oxidise haemoglobin (Hb) resulting in the formation of methaemoglobin (methHb) (Fan et al, 1987; Bruningfann & Kaneene 1993; Knobeloch et al, 2000; ATSDR, 2001). MethHb is a type of Hb that has a reduced ability to transport oxygen (Jaffe, 1981; NRC, 1995) and increased levels of methHb in the blood can greatly reduce the ability of the Hb to carry oxygen from the lungs to the tissues (Jaffe, 1981; NAS, 1995) resulting in methaemoglobinemia (Craun et al, 1981; Kross et al, 1992). The condition is characterised by cyanosis, anoxia, and irregular heartbeat and asphyxiation. The Central nervous system (CNS) effects range from mild dizziness and lethargy to convulsion in affected children (Knobeloch et al, 2000; ATSDR, 2001; Gupta et al, 2008). Cases of this condition have been reported in many countries of the world (including the UK) since its first diagnosis by Comly in 1945 (Ewing &

Mayon-White 1951; Shuval and Gruener, 1972; Knobeloch et al, 2000; Addiscott & Benjamin, 2004), and the current drinking water standard or maximum contaminant level (MCL) of 50mg/l NO_3 was set by WHO in 1958 (WHO, 1958) in order to protect infants from this condition (NAS, 1981; WHO, 1985a). No other health condition was considered (EWG, 1996; Ward et al, 2005).

Recently, IARC reported that, "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is a probable human carcinogen" (IARC, 2010; pg. 39). This classification (Group 2A of IARC cancer classification) was on the basis of limited evidence of carcinogenicity in humans but sufficient evidence of carcinogenicity in experimental animals (IARC, 2010). The mechanism of carcinogenicity by nitrates according to IARC is bacteria reduction of nitrates to nitrites and/-or acidification of nitrites in the stomach to oxides of nitrogen including nitric oxide (NO). NO can undergo nitrosation in the presence of amines and/-or amides in food (endogenous nitrosation) to from N-nitroso compounds (Walker, 1990; Bruning-Fann & Kaneene, 1993; IARC, 2010). Whilst the majority of N- nitroso compounds have been demonstrated to be carcinogenic and teratogenic to animals (NRC 1981; Bogovski & Bogovski 1981; Twort et al, 2000; ATSDR, 2001; Weyer, 2003; IARC,2010) some have been reported to be carcinogenic to human (ATSDR, 2001; Ward et al, 2005; Bjorne 2005; IARC, 2010).

Experimental animal studies suggest that exposure to nitrates can inhibit iodine uptake by the thyroid gland resulting in thyroid disorders (including thyroid cancer) (Bloomfield et al, 1961; 1962; Alexander & Wolff, 1966; Jahreis et al, 1986; Hiasa et al, 1991; Capen, 1997 & 1998), however, possible anti-thyroid effects of nitrates on humans have not been previously evaluated. Given widespread nitrate contamination of drinking water sources in East Anglia and other

regions of the UK, there is a need to evaluate the relationship between nitrate exposure and thyroid disorders (including thyroid cancer) in humans and to quantify any risk in East Anglia. Also, there is a need to evaluate the appropriateness of the current drinking water standard for nitrates (50mg/l) designed to protect against methaemoglobinemia in protecting against thyroid disorders. Nitrate contamination of drinking water and the potential for anti-thyroid effect is a public health issue (Ward et al, 2005) because thyroid hormones are essential for some biological functions such as neurological development; skeletal growth; metabolism and development of the cardiovascular system (Kirk, 2006; Miller et al, 2009) and any disruption of thyroid hormones production can result to adverse health consequences (Crofton, 2008). In a symposium on Nitrogen and Human Health, Powlson et al (2008) called for an independent study to determine whether the current drinking water standard of 50mg/l NO_3 is scientifically justified and/or whether it needs to be reviewed.

It is against this background that this research is undertaken. The study was carried out following the risk assessment framework in order to provide evidence - based risk estimates for thyroid disorders in East Anglia following exposure to nitrates in drinking water. The study involved a systematic search and review of available epidemiological (including case - reports) and experimental animal studies for any evidence of thyroid disorders (including hypothyroidism, hyperthyroidism, goitre and thyroid cancer) due to exposure to nitrates in public and private water supplies and to quantify any excess risk in East Anglia. The study also assessed the extent to which the drinking water standard of 50mg/l NO_3, designed to protect against infantile methaemoglobinemia is likely to protect against thyroid disorders. The findings of this study can contribute to the existing knowledge on the relationship between exposure to nitrates in drinking water and thyroid disorders and also to the debate on the current drinking water standard for nitrates.

1.3 OUTLINE OF THE THESIS

This thesis consists of eight chapters of which this introduction is Chapter One. Chapter Two is an overview of nitrates and drinking water contamination. It also contains information on how nitrates can get into the body (routes of human exposure); what happens to it once inside the body (toxicokinetic) and what it does in the body (toxicodynamics). Chapter Three is the literature review of available experimental animal and epidemiological studies of the anti-thyroid effect of nitrates with reference to PWS and public water. It also contains the result of meta – analysis of relevenat studies as well as the weight of evidence (WoE) analysis. Chapter Four is the study methodology as well as the Aims and Objectives of the study.

Chapter Five is the dose - response assessment between nitrates in drinking water and thyroid cancer. The dose-response assessment was conducted with epidemiological studies only. Chapter Six is the exposure assessment and data analysis and is aimed to determine the amount of water intake by individuals in the study population. Risk characterisation form Chapter Seven. This is the risk estimation stage and incorporates findings from the dose-response analysis and the exposure assessment. It also contains a summary of the uncertainties and assumptions in the risk estimates. Chapter Eight contains a summary of the study findings, the discussion, conclusion and recommendations, including for further research.

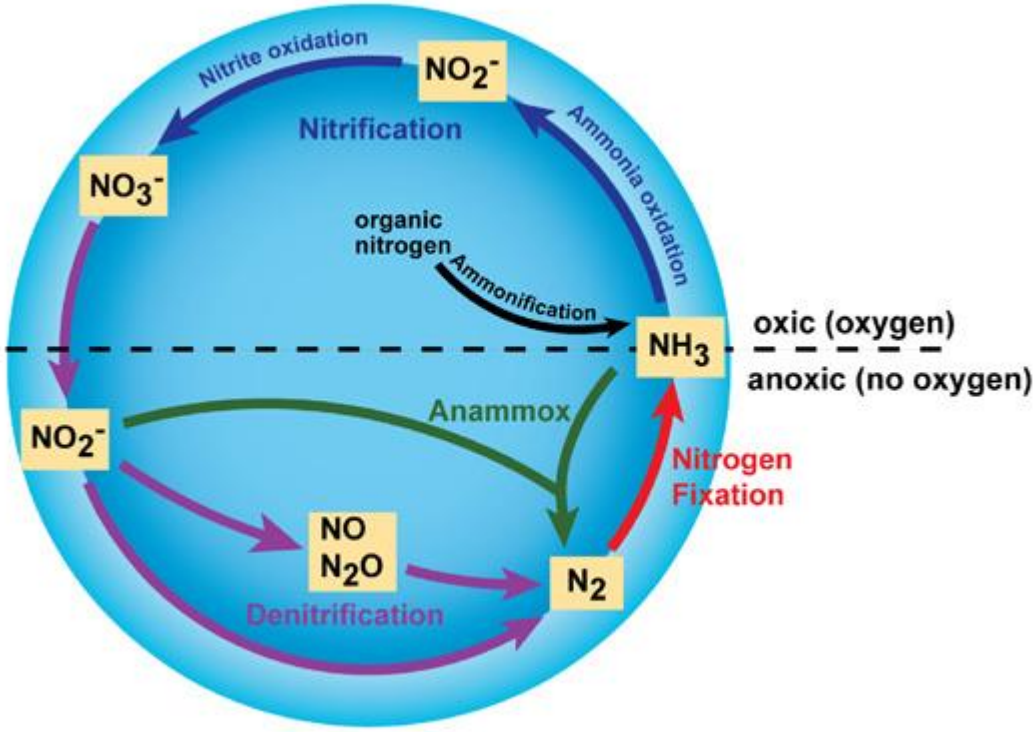
CHAPTER TWO

2.0 NITRATES AND DRINKING WATER CONTAMINATION: OVERVIEW

2.1 INTRODUCTION

Nitrates are freely available in the environment as inorganic ions and are part of the nitrogen cycle (ATSDR, 2001; WHO, 2004a; Manassaram et al, 2006). The nitrogen cycle (figure 2) involves the fixation or conversion of gaseous nitrogen present in the atmosphere (78 per cent) into forms usable to living organisms. The conversion of gaseous nitrogen to nitrate is mediated by free-living bacteria present in plants, soil and water in the presence of sufficient oxygen and to a lesser extent by lightning strikes (Bjorne, 2005). In any environment, nitrate is very stable as oxidised nitrogen and can be reduced to nitrites by microbes (WHO, 2004a). Although nitrate is chemically inert, nitrite is moderately reactive and can further be reduced to various compounds or oxidised to nitrate (WHO, 2004a). Nitrates are present in soils, in most waters and in plants (including vegetables). Due to the stable nature of nitrate ions, most nitrogenous materials in the environment are easily converted to nitrates. Nitrate is mostly used in the manufacture of fertilisers, explosives and in certain pharmaceuticals. Also, nitrates can be used in the chemical industry as oxidising agents and in the food industry as a preservative (WHO, 1978; Bjorne, 2005).

Figure 2 The Nitrogen cycle (Source: Nature Education (2010)).



Anthropogenic activities are the major contributor of nitrates in drinking water sources. Such activities include the use of fertilisers in agricultural activities; sewage discharges; domestic/industrial waste disposal; and emissions from motor vehicles and industries (e.g. power stations). In soil, inorganic nitrogen can first be degraded to ammonia, nitrite and then to nitrate by microbial activities. Given that nitrate is very soluble in water, nitrates in the form of nitrogen based fertilisers applied to crops and not taken up by plants (excess fertiliser) can easily leach from soil to groundwater, removed by surface runoff to rivers or lakes or stored in the soil-water system resulting in the immediate or subsequent pollution of the water course (Van Duijvenboden et al, 1989; WHO, 2004a; Grizzetti et al, 2011).

It is estimated that nitrogen losses from agricultural land account for about 61 per cent of nitrate which enters surface and groundwater water in England & Wales while sewage and other discharges (e.g. livestock production) account for the rest (Hunt et al, 2004; Environment Agency, 2007a). In England, the contribution from agriculture is about 59 per cent, sewage 32 per cent, non- agricultural land and industry accounts for 9 per cent (Hunt et al, 2004). The contribution of agriculture to nitrates in drinking water is greater in more rural regions (e.g. South West, Severn Trent, East Anglia), and smaller in densely populated areas (e.g. Thames) (Hunt et al, 2004). There are two main reasons why agriculture is the major contributor to nitrates in drinking water. The first is the addition of nitrogen in the form of fertilisers to crops. The second reason is because agriculture is the dominant land use, with "managed agricultural land" (i.e. land which receives fertiliser or manures or is cultivated) occupying 61 per cent of the land area of England. A further 14 per cent of the land area is recorded as "rough grazing" (i.e. land which receives no fertiliser or manures) (Hunt et al, 2004; Lord et al, 2006).

The concentration of nitrates in surface water and ground water as a result of leaching from agricultural land in England was 110mg/l and 160mg/l respectively between 2004 and 2006 (lord et al, 2006). Assuming the losses from agricultural land remained stable; it is predicted that the concentration of nitrates in groundwater may reach 200 mg/l in some regions of the UK in a few years (Croll and Hayes, 1988; Lord et al, 2006). There is a strong relationship between nitrate concentrations in groundwater, the amount of nitrogen fertilisers applied to farm land and excess nitrate leaching from farm land (Grizzetti et al, 2011). Higher concentrations of nitrates have been found in groundwater than in surface water (Hunt et al, 2004; Lord et al, 2006) and in sandy soil than in clay soil (Hunt et al, 2004; Lord et al, 2006; Gupta et al, 2008). In groundwater, the presence of nitrates can remain for a very long time even after leaching from soil or farmland has stopped (Spalding and Exner, 1993; USGS, 1999).

2.2 LEGISLATIVE FRAMEWORK FOR MANAGING WATER QUALITY

In the UK, drinking water can be provided either by a statutory water undertaker (Water Company) or privately from private wells or boreholes (PWS). Water companies have a duty under the Water Supply (Water Quality) Regulation 2000 as amended and the Water Supply (Water quality) (Amendment) Regulation 2001; 2002; 2005; 2007) to supply water that is wholesome at the time of supply i.e. when water passes from the water company's pipe into the consumer's pipe. They are responsible for monitoring the quality of their supplies and are also required to make all sampling results available to the Drinking Water Inspectorate (DWI) and the general public via the public register (DWI, 2011).

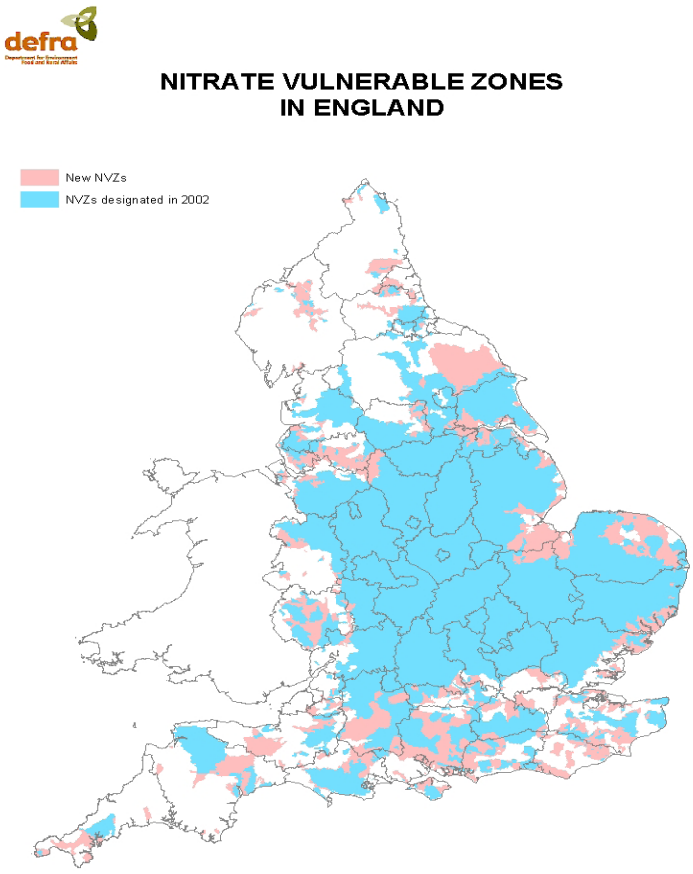
Private Water Supplies (PWS) on the other hand are supplies not provided by a water company (not connected to a mains pipe). In England & Wales, they are regulated by the Private Water Supply Regulation 2009 (previously PWS Regulation 1991). The Regulation (which transpose, implements and enforces the UK's obligation under the revised Drinking Water (Council Directive 98/83/EC of November 3, 1998 on the quality of water intended for human consumption), places a duty on Local Authorities to carry out a risk assessment within five years (from 2009) and to monitor regularly all large and small supplies to ensure that they comply with specified standards. 'Large' supplies are supplies providing water of $10m^3$/day or more (serving 50 or more persons) and/or part of a commercial or public activity (such as bed and breakfast establishments). 'Small' supplies provide water of less than $10m^3$/day (serving less than 50 persons). PWS supplying single dwellings are exempt from the requirement (DWI 2010). According to Article 1 of the EU Directive, 'the objective of this Directive shall be to protect human health from the adverse effects of any contamination of water intended for human consumption by ensuring that water is wholesome and clean' (Council of the European Union,

pg34). Although PWS serving single dwellings are except from the 5 years risk assessment and monitoring requirements, Local Authorities are required by the regulation to carry out risk assessment and monitoring on such supplies only at the request of the owner and or user. The Regulation also specifies monitoring parameters (including nitrate and nitrite), frequency of monitoring, point of monitoring within premises and charging for monitoring. Where quality is found to be below standard, Local Authorities must ensure that necessary remedial actions (including prohibition or restriction of use) are taken to improve the supply (DEFRA, 2005; DWI, 2010).

In order to address and/- or control the pollution of groundwater and surface water especially, that used for drinking water purposes from diffused and point sources, the European Union (EU) has enacted a number of legislations in the form of Directives to Member States for implementation. These Directives have included the Nitrates Directive (Directive 91/676/EEC); the Urban Waste Water Treatment Directive (Directive 91/271/EEC); the Industrial Emission Directive (Directive 96/61/EEC) and the Drinking Water Directive (Directive 98/83/EEC). Also there is the Water Framework Directive (WFD) (Directive 2000/60/EEC) which reinforces the Nitrate Directive and the Urban Water Directive and aims to limit the introduction of nutrients to all waters including inland, surface coastal waters and groundwater. The Groundwater Directive (Directive 2006/118/EEC) complements the WFD and is for the protection of aquifers from pollution. Whilst all these legislations were aimed at reducing the nutrient load to surface water, groundwater, coastal and marine waters, the most relevant Directives to this study are the Nitrates and Drinking Water Directive and the Drinking water Directive.

The Nitrates Directive aims to reduce nitrates from agricultural lands entering and polluting water courses and to prevent such pollution in the future. It requires Member States to designate as NVZs all land draining to waters that may be affected by nitrate pollution. Within a NVZ, mandatory restrictions are placed on the use of organic manures and inorganic fertilisers on agricultural land (Environment Agency, 2007a). These measures were expected to reduce nitrate concentrations in limestone and sandstone aquifers over time (Tebbutt, 1998). In implementing the Nitrates Directive; the UK government designated some land in different regions of the country as Nitrate Vulnerable Zones (NVZs). Figure 5 shows NVZs in England including the study area, East Anglia.

Figure 3: Map showing Nitrate Vulnerable Zones in England (Source: DEFRA, 2009).



The map show that about 77 per cent of agricultural land in England has been designated as NVZs. Whilst about 22 per cent of land was designated in 1996, a further 55 per cent was designated in 2002 (DEFRA, 2009). The NVZs included the majority of land in East Anglia (DEFRA, 2009), where land use other than for residential purposes is predominantly (>75 per cent) used for agriculture.

2.3 NITRATE CONCENTRATIONS IN DRINKING WATER SOURCES IN THE UK

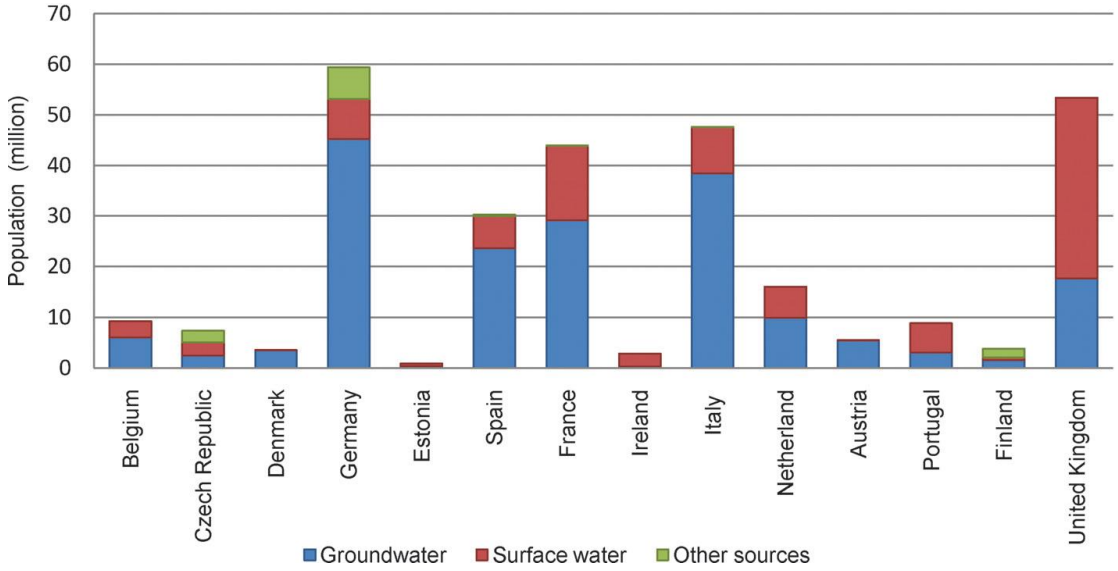
Nitrates are naturally present in ground and surface water and depending on soil type, background levels are usually less than 10mg/l in groundwater and <5mg/l in surface water (Meybeck & Helmer, 1996; European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), 1988). However, any exceedance of these levels is an indication of pollution from varying sources (WHO, 2004a). Nitrate contamination of water sources in the UK has increased since the 1950s due mainly to agricultural practices (especially the application of nitrogen based fertilisers or manure to crops) and other land uses in the catchment (Environment Agency, 2007a), with annual average increases of about 0.7mg/l recorded in some surface water (Young & Morgan- Jones, 1980). Although the concentration of nitrate in surface water varies from day to day, high concentrations have been reported in dry areas or regions such as East Anglia when compared with wetter areas or regions. This is because, in wetter areas, the rate of dilution is faster than in dry areas (Environment Agency, 2007). Nitrate concentration is also higher in arable areas (including East Anglia, the Midlands and Thames) than in grassland areas due to high rate of microbial degradation of nitrates (Environment Agency, 2007).

The Environment Agency conducted an assessment of nitrate concentrations in surface water and groundwater in England between 1999 and 2004 and reported that although concentrations had reduced in some areas, concentrations are still high in some areas or regions such as East Anglia (Environment Agency, 2007). While data on nitrate concentrations in both surface water and groundwater showed that nitrates concentrations in surface water were higher compared to groundwater, the long time it takes for nitrates leaching from agricultural land to reach groundwater when compared with surface water may explain the reason. Although the mean concentration of nitrates in water in some regions was low, there were differences in concentration between sites in the same region and between regions (Environment Agency, 2007).

In the European Union (EU), groundwater accounts for the majority of drinking water in many EU15 countries and is the main source of drinking water in countries such as Austria, Denmark, Italy, Spain, Germany, France and the Netherlands while surface water is dominant in the UK, Finland, Czech, Estonia, Ireland and Portugal (European Commission, 2007). Data from the European Commission (2007) suggest that between 2002 and 2004 the contribution of surface water, groundwater and other (e.g. bottled water) to drinking water was 66; 33 and 4 per cent respectively. Figure 6 shows the contributions of surface water and groundwater to drinking water and the percentage of the population served in the EU14 between 2002 and 2004 suggesting that groundwater contributed drinking water for less than 20 per cent of the population in the UK while surface water contributed drinking water for the majority of the population. About one- third of these water sources are estimated to contain nitrates in excess of the drinking water standard of 50mg/l (European Commission, 2002; WHO, 2004b). According to data made available to the European Commission in compliance with the Drinking Water Directive 98/83/EEC which requires Member States to report on drinking water quality every

three years, nitrate was one of the parameters that usually failed to meet the required standard in the 2002-2004 assessment (European Commission, 2007).

Figure 4: Relative Contribution of Surface Water, Groundwater, and other sources of Drinking Water in EU14. Source: European Commission, (2007)



Although data on nitrate concentrations in drinking water made available to the European Commission only related to public supplies and from large supply zones serving more than 5000 persons or producing over 1 million litres of water per day (European Commission, 2007), high nitrate concentrations were also been reported in small supplies (PWS) (where the mandatory reporting contained in the Drinking Water Directive does not apply) in the Netherlands, Belgium, Germany, UK, France, Denmark and Slovenia (Strebel et al, 1989; MAFF, 1992; Fried, 1991; Maticic, 1999; WHO 2004b), with levels in private wells about 10-15 times higher than the drinking water standard (Van Duijvenboden & Mattijesen, 1989). In Denmark, about 30

per cent of the population rely on PWS for their drinking water while the percentage in Austria, France, Germany and Ireland is about 25 per cent (European Commission, 2007). In the UK, about 1.6 per cent of the population in England is permanently served by PWS with a further 7.8 million people occasionally exposed to water from PWS (DWI, 2013). These private-wells, which are mostly located in rural areas and areas of intensive agriculture and crop production, are sometimes shallow when compared to public supply boreholes and therefore more easily contaminated by nitrate and other water contaminants than public water supply boreholes which are usually located in deeper groundwater aquifers (USGS, 1996a; Maticic, 1999).

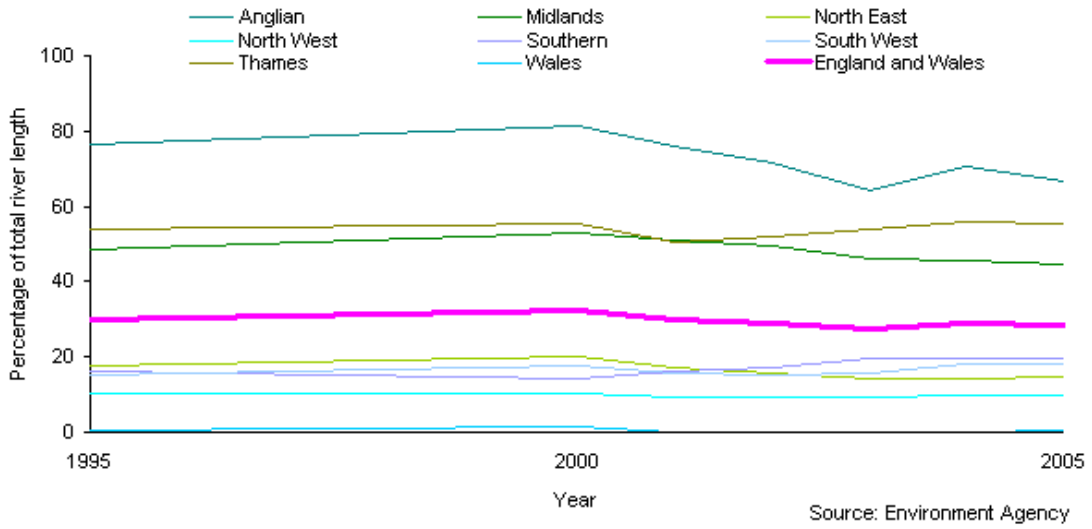
In the EU27, Grizzetti et al, (2011) analysed European Commission data and estimated that about half of the population live in areas where nitrate concentration in both groundwater and surface water is higher than 25mg/l while about 20 per cent live in areas with levels higher than 50mg/l. The data suggest that in the UK, about 38 per cent of the population live in areas with nitrate concentrations in drinking water of <25mg/l while about 22 per cent live in areas with >50mg/l. About 30 per cent of the population live in areas with 25-40mg/l while about 10 per cent live in areas with 40-50mg/l. In the Netherlands, only about 5 per cent of the population live in areas with nitrate concentration less than 25mg/l while about 52 per cent live in areas with nitrate levels in drinking water >50mg/l.

2.4 TRENDS IN NITRATE CONCENTRATIONS IN THE UK

Nitrate concentrations in surface water fluctuate from year to year and consequently, there is no clear national trend (Environment Agency, 2007). This fluctuation is mainly due to changes in

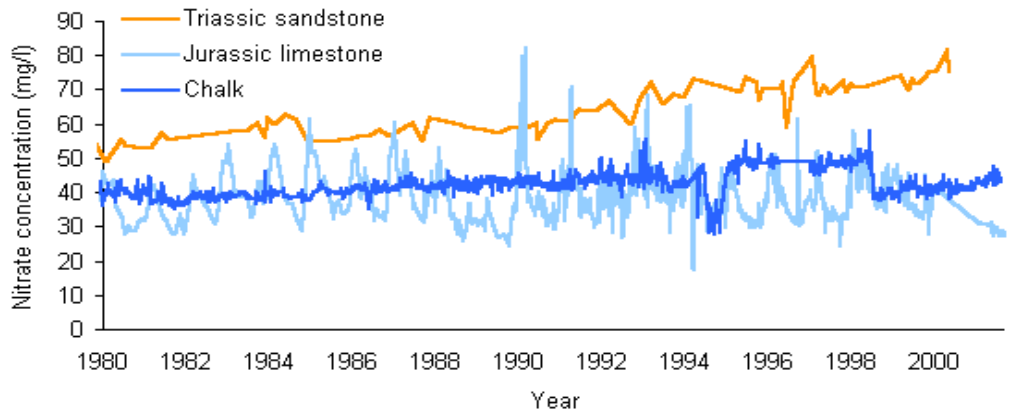
nitrogen input from agricultural activities; sewage treatment works and discharges from industries, as well as seasonal variations, with concentration higher in winter (due to high runoff after a dry summer when nitrogen has built up in the soil either from fertilisers or deposition from the air) and lower in summer. Analysis of nitrate concentrations in surface water in England &Wales by the Environment Agency between 1999 and 2004 showed that nitrate concentration was increasing in about 77 per cent of sites and decreasing in only about 23 per cent of the sites (Environment Agency, 2007), although this increasing and decreasing trend was only statistically significant in a few cases and this may be as a result of the short period of time over which the analysis took place. However, a look at a longer time period between 1995 and 2005 (figure 8) shows that 28 per cent of rivers had high nitrate concentrations in 2005 (mean concentration >30mg/l), compared with 32 per cent in 2000 and 30 per cent in 1995 (Environment Agency, 2007). A mean of 30mg/l was used as a basis for the assessment to indicate the potential for nitrate concentration to exceed the drinking water standard of 50mg/l at some time in the future (Environment Agency, 2007). Although these figures suggest a slight downward trend, they also indicate that there are variations between and within regions, as well as seasonal variations (Environment Agency, 2007).

Figure 5: Percentage of rivers with high nitrate concentration in England & Wales 1995-2005



In a groundwater nitrate assessment, the Environment Agency (Environment Agency, 2007) observed a trend more rising than falling in groundwater monitoring points. According to the Agency, nitrate concentrations in groundwater depend on farming practices as well as the aquifer type (Environment Agency, 2007). While it may take decades for water to move through some aquifers with the result that pollution will remain in the aquifers for a long time, other aquifers respond more quickly and the retention time within the aquifer is shorter. Figure 8 represents data from the Environment Agency on groundwater nitrate concentrations in several aquifer types suggesting that nitrate levels in some groundwater had doubled since 1980 with the Triassic sandstone and Jurassic limestone nitrate concentrations rising well above the regulatory limit of 50 mg/l NO_3 (DEFRA, 2004). It is estimated that about 45 per cent of groundwater in England is at the risk of nitrate contamination.

Figure 6: Trends in groundwater nitrate concentrations in England and Wales, 1980-2001 (Environment Agency, 2006).



Nitrate concentrations at three representative sites Source: Environment Agency

Nitrate contamination of groundwater is still a concern in Europe given that about two- thirds of Europe's population rely on groundwater for their drinking water (Grizzetti et al, 2011).

According to data released by the European Commission (EC (2007) COM (2007) Report 120) in the implementation of the Nitrate Directive, about 17 per cent of wells in EU15 had nitrate concentrations above 50mg/l between 2000 & 2003. In the same period, about 7 per cent of the stations had nitrate concentration between 40-50mg/l, while about 60 per cent had concentrations below 25mg/l. Although the figures suggest improving trends in about 30 per cent of the wells, concentration remained high in about 36 per cent of the wells (EC (2007) COM (2007) Report 120). According to the report covering the period between 2004 to2007, nitrates concentration was still high in about 34 per cent of the wells, with about 15 per cent containing nitrates above the drinking water standard of 50mg/l (European Commission (2010) COM (2010) Report 47).

Despite efforts by means of legislation and conventions to reduce nitrate contamination of drinking water in Europe, including the UK, the rising trend in nitrate pollution of drinking water sources (including groundwater and surface water) suggests that anthropogenic activities continues to impact on drinking water sources and will continue to do so in the future. The high but stable nitrate concentrations in Western Europe, the large reserves of nitrogen that have already built up in soil and aquifers and the potential for intensive agriculture and increased use of fertilisers in crop production in Eastern Europe in the coming years will likely remain a threat to water quality and it is not known how long it will take for drinking water quality to be restored in affected countries (Grizzetti et al, 2011). According to the UK Environment Agency, current legislations are unlikely to reverse past nitrate pollution or prevent the rising trends of nitrate concentrations in groundwater, and it is unlikely that reductions in nitrate concentrations in surface water will be achieved in the near future (Environment Agency, 2007a). Therefore it is necessary to assess the potential ecological and public health impact of rising trend in nitrate concentrations in drinking water sources in order to determine any mitigation measures that need to be put in place for public health protection.

2.5 ROUTES OF EXPOSURE

Humans can be exposed to nitrates exogenously from dietary and drinking water sources. Whilst vegetables such as potatoes, lettuce, radishes, and spinach are the main sources of dietary nitrates (Chilvers et al, 1984; USEPA, 1987; ECETOC, 1988; WHO, 2004a), other dietary sources include cured meat, processed cereal products, some preservatives used in the food industry (WHO, 2004a), as well as the use of aluminium products in food preparation which can reduce nitrate to nitrite, leading to increased toxicity (WHO, 2004a). Although vegetables constitute the main source of nitrate exposure when nitrate concentrations in drinking water is below 50mg/l (IARC, 2010), drinking water can be a major source of nitrate exposure in areas where nitrate concentration are above the drinking water standards of 50mg/l (Chilvers et al, 1984; WHO, 2004a), accounting for about 80 per cent of total nitrate intake especially for bottle-fed infants (Chilvers et al, 1984; WHO, 2004a). Although nitrate is not toxic per say, its reduction to nitrite and then to nitric oxide by bacteria present in the gastrointestinal tract results in its toxicity. Nitrite is rarely present in drinking water (WHO, 2004a), but when present, the concentration is usually below 3mg/lNO_2^- and is an indication of bacterial contamination of drinking water in the distribution system (nitrite is produced from ammonia by ammonia- oxidising bacteria) due to inadequacies of the chloramination disinfection process (WHO, 2004a; Forman, 2004). Chloramination is the process of water treatment for public health protection where chlorine and a small amount of ammonia are added to drinking water at different times as disinfectants (USEPA, 2007).

Air pollution can contribute to human exposure to nitrates. This can be from inhalation of cigarette smoke and car exhausts (Health Canada, 2013). Although air pollution can contribute to

nitrate exposure, its contribution is minute when compared with dietary and drinking water sources (WHO, 2004a). Therefore exposure through the oral route is very significant in the risk assessment of nitrates (Lunberg et al, 2004). There is no evidence of nitrate or nitrite exposure through the dermal routes (Health Canada, 2013). The effect of nitrate exposure is usually the same whether nitrate containing compounds are ingested, inhaled or produced endogenously (ATSDR, 2001).

2.6 TOXICOKINETICS

Toxicokinetics has to do with the movement of a toxicant around the body in the process of absorption, distribution, metabolism and elimination or excretion (Trush, 2008). In other words, it refers to what the body does to the toxicant at every stage of its movement and is indicated by the concentration of the toxicant in the plasma at various stages, leading to a biological effective dose of the toxicant at the end of the process (Trush, 2008).

2.6.1 ABSORPTION AND DISTRIBUTION

In humans, absorption of ingested nitrate takes place in the upper part of the small intestine from where it combines with endogenously synthesised nitrate in the bloodstream (Balish et al, 1981; Bartholomew & Hill, 1984; Bjorne, 2005). The background concentration of nitrate in the bloodstream is about 20-30µmol/l but this may increase 20-40 folds within 2 hours following exposure to nitrate from exogenous sources (Mcknight et al, 1997; Lundberg & Govoni, 2004).

Following ingestion, approximately 60-70 per cent of nitrates is excreted in urine in the first 24 hours, while about 25 per cent is secreted in the saliva by blood active transport mechanism where about 5-10 per cent is reduced to nitrite by bacteria usually present at the back of the tongue (Spiegelhader et al, 1976; Duncan et al, 1995; Bjorne, 2005), but this value may be up to 20% in some individuals (Spiejers & Brandt, 2003; Weitzberg & Lundberg, 1998) especially the elderly (Eisenbrand et al, 1980). Microbial reduction of nitrate to nitrite in the oral cavity is influenced by nutritional status, infection, temperature and age (Eisenbrand et al, 1980). From the oral cavity, nitrite and the remaining nitrate are then swallowed in saliva to enter the stomach (Lundberg & Weitzberg, 1998; WHO 2004a). The concentration of nitrate or nitrite in the saliva is related to the amount of nitrate ingested (Spiegelhader et al, 1976; Bartholomew & Hill 1984). Although the reduction of nitrate to nitrite can occur in other parts of the gastrointestinal tract, it does not usually occur in the stomach because of the presence of gastric acid (low pH) (Colbers et al, 1995). However in individuals with little or no gastric acid e.g. bottle fed infants or people with diarrhoea, bacteria colonisation of the stomach can occur leading to high pH and consequent reduction of nitrate to nitrite by micro-organisms (WHO, 2004a). Salivary secretion and reduction of nitrate to nitrite have also been reported in experimental animals like rats (Duncan et al, 1995) but the total nitrate reduction is less than that in humans (Health Canada, 2013; WHO, 2004a).

2.6.2 METABOLISM AND BIOTRANSFORMATION

In the stomach and other acidic environments (like the ischemic heart; urine; mouth and skin), nitrite can acquire a proton (H^+) to from nitrous acid (HNO_2) which can subsequently yield various species of nitrogen compounds such as dinitrogen trioxide (N_2O_3), nitrogen dioxide

(NO_2) and nitric oxide (NO) (Addiscott & Benjamin, 2004; Bjorne, 2005; Lundberg & Weitzberg, 2005). This process of acidification of nitrite to yield various species of nitrogen oxides is enzyme independent; however, the presence of reducing agents can favour the production of more NO (a less reactive nitrogen oxide) than NO_2, N_2O_3, N_2O_5 (Bjorne 2005). According to Bjorne (2005), acidification of nitrite is a major source of nitric oxide in humans and can sometimes produce nitric oxides in excess of that required for the maintenance of normal biological cell functions. Apart from the acidification of nitrite to various species of nitrogen oxides, NO can also be produced at neutral pH by bacteria reduction of nitrite (Ohshima and Bartsch, 1994).

2.6.3 ENDOGENOUS NITRATE SYNTHESIS

Nitrate is also produced endogenously (Walker, 1995) and it is estimated that about 1mmol (62mg) of nitrate is produced endogenously by a healthy adult human per day (Wishnok et al, 1995). The main pathway for endogenous nitrate synthesis is thought to be through the L-arginine - nitric oxide (NO) pathway (Tjeert et al, 2003; Bjorne 2005; IARC, 2006). This process is mediated by the enzyme, nitric oxide synthase (NOS) in the presence of oxygen and is dependent on nicotinamide adenine dinucleotide phosphate (NADPH). NOS is present not only in microphages and neutrophils but also in all mammalian cells including the epithelium of the gastric mucosa (Bjorne, 2005). This pathway involves the production of nitric oxide and L-citrulline from the amino acid, L-arginine. This is then followed by the oxidation of nitric oxide (NO) to nitrogen dioxide (NO_2) and dinitrogen trioxide (N_2O_3). N_2O_3 can react with water to yield nitrous acid (HNO_2) which can dissociate to nitrite and H^+. Nitrite is easily oxidised to nitrate through reaction with the haemoglobin (Addiscott & Benjamin, 2004; IARC, 2010). The

excess nitrate detected in urine following exposure to low levels of nitrate or nitrite is suggests endogenous synthesis of nitrate (Leaf, 1989). Gastrointestinal infection, respiratory disease or inversion of the body by micro-organisms is reported to increase endogenous production of nitrates in order to protect the body from invading microbes (Green, 1981; Bartholomew & Hill, 1984; Gangolli et al, 1994). Thus in situations of low exogenous nitrate exposure, endogenous nitrate production are a major source of nitrates in the body (Mensinga et al, 2003). Although the toxicological consequences of endogenously produced nitrates are not yet known (Wishnok et al, 1995), it however, makes nitrate risk assessment difficult (Spiejers, 1998; WHO 2004a).

2.6.4 EXCRETION

The half-life of nitrate in the body is about 5-7 hours (Green et al, 1981; Spiejers, 1998) and complete after 24hours (Bartholomew & Hill, 1984). Whilst the majority (60-70 per cent) of ingested nitrate is excreted in urine as nitrate, ammonia or urea, approximately 1 per cent is excreted in faeces (Bartholomew & Hill, 1984; Gupta et al, 2008), and the remainder is either excreted in sweat, exhaled in breath (L'hirondel & L'hirondel, 2002); Bjorne, 2005). Approximately 25% of nitrate is actively transported by the sodium/iodide symporter to saliva and breast milk (Wagner et al, 1983; Walker, 1999). In infants, approximately 100 per cent of ingested nitrate is excreted in urine under normal conditions (Turek et al, 1980). The half-life of nitrite is approximately 30 minutes in humans and as a result is not normally detected in body tissue or fluid after oral exposure (Kortboyer et al, 1997). Only a very small amount of nitrite is excreted in urine (WHO, 1985b; Speijers et al, 1989; Gupta et al, 2008) as a result of bacteria reduction of nitrate to nitrite in the urinary tract (Mensinga et al, 2003). The half-life of NO_x is about 1-5 seconds due mainly to its rapid reaction with oxyhaemoglobin resulting in

methaemoglobin and oxidation to nitrate (Bjorne, 2005) and reaction with amines to form nitrosamines (Health Canada, 2013).

2.7 TOXICODYNAMICS

Toxicodynamics refers to the actions of a toxicant or their metabolite on biological systems as a result of the interaction of the biologically effective dose with a molecular target (Trush, 2008). The biological effect of nitrates (including its toxicity) is mediated by its metabolite NO_x through the reduction of nitrates to nitrite and then to oxides of nitrogen including, NO (Addiscott & Benjamin, 2004; Bjorne, 2005). Nitric oxide is also known to be biologically active and can play a role in some biological functions (Jaffe, 1981; Hsia, 1998). Such functions include vasodilation (Bjorne, 2005), neurotransmission (Garthwaite, 1991), antimicrobial activity (Hibbs et al, 1987; Fang, 1997; Addiscott & Benjamin, 2004); immune regulation (Hibbs, 1991), vascular smooth muscle relaxation (Ignarro, 1989) and inhibition of platelet aggregation (Randomski et al, 1987; Bjorne, 2005). However, nitric oxides can also be toxic to cells (including tumour cells) if produced in large amount e.g. following ingestion of large amount of nitrates (Gupta et al, 1998) or if any of the cell protective mechanism is compromised (Sutton et al, 1976; Hsia, 1998; Al-Sa'doni & Ferro, 2000). NO_x can also react with secondary and tertiary amines or amides such as nitrosamines and nitrosamides to from N-nitroso compounds (NOCs) (Butler and Williams, 1993; Wink & Mitchell, 1998; Baker et al, 2004). The majority of N- nitroso compounds (about 80 per cent) are carcinogenic to animals (Choi, 1985; De Roos, 2003; Gupta et al, 2008) while some are carcinogenic to humans (IARC, 1978). Endogenous nitrosation accounts for about 75 per cent of the overall human exposure to N-nitroso- compounds while exogenously formed

NOCs found in preserved meat, fish, tobacco products and certain occupational exposures accounts for the rest (Tickner, 1997).

Although methaemoglobinemia is outside the scope of this study, NO_x is reported to be responsible for the oxidation of oxyhaemoglobin to methHb (Addiscott & Benjamin, 2004). Also the reaction of NO (at high concentration) with oxygen radicals (O_2^-) can result in the formation of peroxynitrite ($ONOO^-$) (Addiscott & Benjamin, 2004; Al-Sa'doni & Ferro, 2000; Bjorne, 2005). Peroxynitrite has been implicated in the oxidation of thiols; DNA damage (Gupta et al, 1998; Bjorne, 2005); lipid peroxidation (Al-Sa'doni & Ferro, 2000), and mitochondria dysfunction (Gupta, 1998, Bjorne, 2005).

2.7.1 MODE OF ACTION OF NITRATES ON THE THYROID GLAND

The mode of action of nitrate on thyroid is reported to be through its ability to inhibit iodine uptake by binding to the sodium/ iodine symporter (NIS), a glycoprotein membrane located on the basal side of thyroid, resulting in decreased thyroid hormones (triiodothyronine (T3), thyroxine (T4)) production; and increased level of thyroid stimulating hormones (TSH) (Bloomfield et al, 1961 & 1962; Alexander & Wolff, 1966; Greer et al, 2002). Chronic stimulation of the thyroid gland to produce more hormones by the TSH in the event of low thyroid hormones can result to thyroid disorders, including hypothyroidism; increased thyroid volume; thyroid hypertrophy (goitre); hyperplasia; adenomas and carcinoma (Hiasa et al, 1991; Capen, 1997 & 1998). High TSH is a biomarker for hypothyroidism while altered T4 serum concentration is a biomarker for exposure to endocrine disrupting chemicals (De Vito et al, 1999;

Zoeller et al, 2007). Whilst it is not known the level of thyroid hormones below which adverse thyroid effects can occur, available evidence suggests that the half- life of serum thyroid hormones (T4) in adult humans is 7-10days (Vulsma et al, 1989; Greer et al, 2002) and approximately 3days in infants (Vulsma et al, 1989). In rats, the half-life of thyroid hormones is approximately one day (Zoeller & Croffton, 2005). However, while adult humans are able to store several months' worth of thyroid hormones to be used when there are shortages (Greer et al, 2002), it is estimated that infants can only store less than one day worth of thyroid hormones (Zoeller & Croffton, 2005). The short half –life of thyroid hormones in rats and infants implies that they require more iodine intake to produce more hormones. This therefore suggests that rat and infants and are more sensitive to iodine inhibitors than adult human (Health Canada, 2013). Although adult animals and humans can compensate for shortages of thyroid hormone production from their reserve if there are shortages of iodine uptake by the thyroid gland, however, exposure to high levels of nitrates or nitrites; chronic exposure or combined exposure with other iodine disrupting chemicals, coupled with dietary iodine deficiency can also result in the rapid depletion or exhaustion of reserved thyroid hormones, resulting in hypothyroidism and/-or thyroid hypertrophy (goitre) (Health Canada, 2013). Hypothyroidism is also very common during pregnancy as it adds pressure on the thyroid gland (Aoki et al, 2007).

The NIS is also present in other tissues like the mammary gland and acts as a source of iodine transport from mother to child during lactation (Kirk, 2006). Adequate thyroid hormone production is necessary for neurological development, skeletal growth and normal function of the pulmonary and cardiovascular systems; metabolism; kidney and serum lipids functions (Kirk 2006; Miller et al, 2009). Therefore, inadequate or insufficient thyroid hormones can result in developmental defects in animals and human as a result of decreased tissue T4 or T3 irrespective of the level of TSH (Crofton, 2008). Decreased thyroid hormones (even when clinical

hypothyroidism is not evident) and especially during critical or sensitive periods of development can result in impaired foetal development, reduced heart rate and body heat producing capabilities and reduced intelligent quotient (IQ) (Howdeshell, 2002; Kirk, 2006). Although disruption of thyroid hormone production can result in thyroid tumours and birth defects (Health Canada , 2013), it is not well established if humans can get carcinoma from thyroid hormones deficiencies given that humans thyroid cells are less susceptible to the effects of TSH than rats (Crofton, 2008). Although other drinking water contaminants like perchlorate and thiocyanate can inhibit iodine uptake by the thyroid, the relative potency of perchlorate to inhibit radioactive iodine uptake has been found to be 15,30,240 times that of thiocyanate and nitrates respectively (Tonacchera et al, 2004). Based on the molar potencies, DeGroef et al (2006) suggested that thiocyanate and nitrate acquired through food and drinking water accounts for a much larger proportion of iodine uptake inhibition than perchlorate. Although nitrate from food and drinking water are both reduced to nitrite and NO, nitrates from food sources including vegetables are unlikely to result in increased endogenous nitrosation because of the presence of antioxidants such as ascorbic acid (Vitamin C), alpha- tocopherol or other reducing agents normally present in vegetables and food which can inhibit endogenous nitrosation (Bartsch et al, 1988; IARC, 2010). No association has been reported between dietary nitrate intake and human cancers and this has been attributed to the presence of antioxidants and nitrosation inhibitors normally present in vegetables and food (Boeing, 1991; Forman, 1987; Ward et al, 1996). NIS does not transport nitrite (Eskandari et al, 1997) suggesting that the toxicity or effect of nitrates on the thyroid gland is not mediated by nitrite

However, exposure to elevated levels of nitrates in drinking water can result in increased production of nitrite and NO_x (Cole & Brown, 1980) and increased endogenous nitrosation (Ward et al, 2005; Gupta et al, 2008,) due to the absence of antioxidants in drinking water

(IARC, 2010). It has been reported that exposure to elevated levels of nitrate in drinking water above the drinking water standard (>50mg/l) is associated with the ability to nitrosate proline, a biomarker for endogenous nitrosation in urine (Moller et al, 1989; Mirvish et al, 1992); and increased concentration of N-nitroso compounds in faeces (Rowland et al, 1991; Chiu et al, 2007). Below 50mg/l, no relationship between nitrate exposure and formation of NOCs was reported (Levallois et al, 2000). However, at 50mg/l, exposure can result in the formation of NOCs if nitrosatable compounds are also present (Vermeer et al, 1998). According to Shepherd (1995); WHO (1996b); endogenous nitrosation can occur in the gastric juice of humans mostly at low pH when either NO; NO_2; N_2O_3 or N_2O_4 and nitrosatable compounds are present at the same time.

Given that antioxidants are not normally present in drinking water (IARC, 2010) exposure to high concentrations of nitrates from this medium is a public health concern (Ward et al, 2005). This is because water is likely to be consumed without simultaneous exposure to antioxidants. It is for this reason that this study only evaluated exposure to nitrates from drinking water. According to IARC (2010), evaluation of nitrate exposure from food and drinking water can be conducted separately because of the presence of antioxidants in food which can inhibit nitrosation. Single medium risk assessment is justified if the levels of contaminants present in a medium exceeded the guideline value or when required by legislation (Davis & Klein, 1996; DEFRA, 2009).

2.8 SUMMARY

Nitrate contamination of drinking water sources is widespread in parts of the UK especially in the South West; East Anglia and the Midlands. Agricultural processes especially the application of fertilizers to crops is a major source of nitrate pollution of drinking water sources. Nitrate contamination of surface water and groundwater used for drinking is mainly due to human activities especially the use of nitrogen based fertilizers and manure in crop production. Nitrate concentrations in drinking water sources vary, but there is consistency in the relationship between nitrate concentrations and sources of water. Thus, higher nitrate concentrations have been found in groundwater than in surface water; in private water supplies than in public water; in shallow wells than in deeper wells; and in agricultural areas than urban areas. High nitrate concentrations especially in PWS is a public health issue because unlike public water, this type of supply are more susceptible to pollution because they well are not as deep as public water boreholes and users of such water are more likely to be exposed to nitrates in drinking water above the WHO drinking water standard.

Humans are exposed to nitrates from both exogenous and endogenous sources. Whilst the contribution of endogenously synthesised nitrate to the overall nitrate body burden is very small and may be toxicologically insignificant, exogenous sources are the main contributors of nitrate exposure to humans. Given that the biological effect of nitrates is mediated by nitric oxides (nitrates is reduced to nitrites and then to NO) and not nitrite as previously thought, exposure to high concentrations of nitrate in drinking water is associated with increased production of NO. Although NO can play a role in the maintenance of biological functions, it is also cytotoxic and can result in the inhibition of iodine uptake by the thyroid gland. This could result in in low production of thyroid hormones and increased production of TSH. Chronic stimulation of the

thyroid by TSH to produce more hormones in the event of shortages could result in hypothyroidism, increased thyroid volume, thyroid hypertrophy (goitre), hyperplasia and carcinoma. NO and other oxides of nitrogen such as N_2O_3; N_20_5 and NO_2 can also undergo nitrosation in the presence of secondary or tertiary amines or amides to form N-Nitroso-compounds. Whilst the majority of NOCs are known animal carcinogens, some have been reported to be carcinogenic to humans.

Although food, especially vegetables is the main source of exogenous nitrate exposure in humans, it is less likely to increase endogenous nitrosation because vegetables contain antioxidants e.g. vitamins C, E and alpha - tocopherol which can inhibit endogenous nitrosation. However, exposure to nitrates in drinking water could result in increased endogenous nitrosation since antioxidants are absent in water and water is more likely to be consumed without concomitant exposure to any antioxidant. Drinking water is therefore a major source of exposure to nitrates and a justification of the single medium risk assessment.

CHAPTER THREE

3.0 LITERATURE REVIEW

3.1 INTRODUCTION

The purpose of this chapter is to systematically review the literature (epidemiological and experimental animal studies as well as case – reports) for evidence of thyroid disorders as a result of exposure to nitrates in drinking water. It involves characterising the nature of any disorder (e.g. hypothyroidism; hyperthyroidism; goitre; thyroid cancer) and the strength of any evidence of association and determining whether any association is causal. The Chapter therefore aims:

- To review available epidemiological and experimental animal studies as well as case-reports for evidence of thyroid disorders following exposure to nitrates in drinking water.
- To characterise the mechanism of action of nitrates on the thyroid gland.
- To characterise the nature of any disorder as well as the strength of any evidence of association.
- To determine whether any association is causal.

Systematic review is very important in healthcare and aims to provide information that can be used to bridge knowledge gaps and aid policy formulation, and may also form the basis for future research. It is therefore important that the process is rigorous and follow prior planning and documentation of the methodology or protocol (Shamseer et al, 2015). According to Shamseer et al, (2015), the reasons why a protocol for systematic review is important are:

- To enable other reviewers to replicate the review methods if necessary and to judge the validity of planned methods.
- To prevent arbitrary decision making with respect to inclusion criteria and extraction of data.
- To reduce duplication of efforts and enhance collaboration

The PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analysis) framework has been designed and recommended for systematic review and meta-analysis. The PRISMA protocol (PRISMA-P) presents an explicit scientific road map of a planned systematic review. It details the rationale and planned methodological and analytical approach of the review (Moher et al, 2009; Shamseer et al, 2015). This review was conducted following the PRISMA framework.

The literature reviewed in this thesis was obtained by a systematic search of the following online databases:

- PubMed (www.ncbi.nlm.nih.gov)
- Toxline (Toxline Database, National Library of Medicine, Bethesda, MD (www.toxnet.nlm.nih.gov)
- ScienceDirect (www.sciencedirect.com)
- ISI Web of Knowledge (www.isiknowledge.com)
- Google Scholar (http://scholar.google.com)

Given that the mode of action (section 2.7.1) suggest that exposure to nitrates can inhibit iodine uptake by the thyroid resulting decreased thyroid hormone production, hyperthyroidism, hyperthyroidism, thyroid hypertrophy (goitre) hyperplasia and carcinoma, the following key words were used: (“nitrates”) AND (“drinking water”) AND (“thyroid disease”) OR (“thyroid

disorders"). Additional searches were also conducted using the search term: ("nitrates") AND ("drinking water") AND ("thyroid neoplasms") OR ("thyroid cancer") OR ("goitre"). Papers were obtained if they had nitrates; nitrite; drinking water and thyroid disorders (including thyroid cancer, hypothyroidism, hyperthyroidism and or goitre in their title or text). A sample of the search term and number of papers obtained through online search or databases is as in Appendix 1. In addition, hand searches were also conducted on library resources for relevant papers, books, abstracts and conferences proceedings. Some papers were also obtained from the "grey" or "fugitive" literature and reviewed in order to reduce publication bias as well as improve the comprehensiveness of this review (McAuley et al, 2000). Bibliographies of papers retrieved were checked for relevant additional references and these were also obtained. The inclusion criteria included:

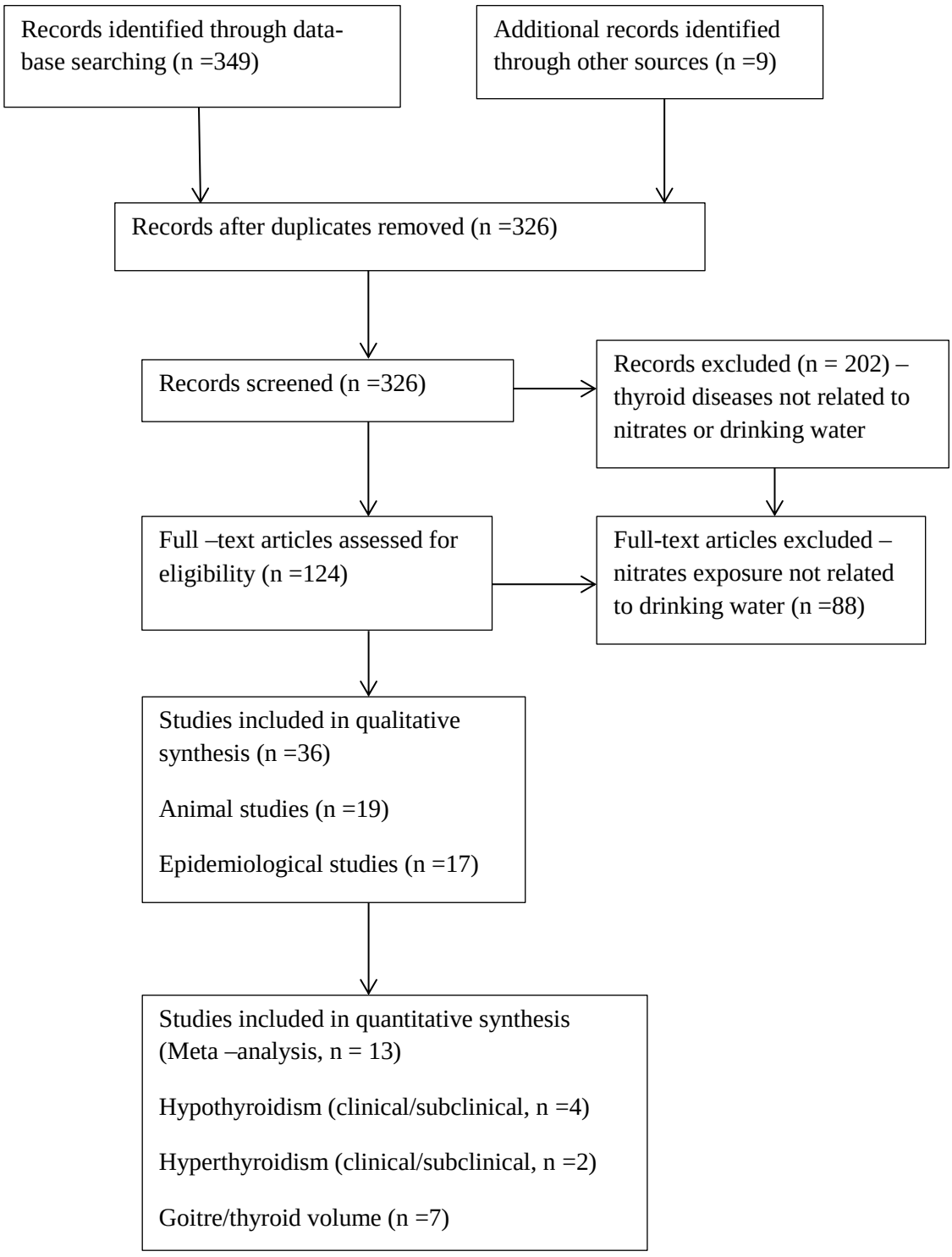
- Animal studies demonstrating thyroid disorders following exposure to nitrates in drinking water.
- Epidemiological studies (including case-control, cohort and ecological studies) investigating the relationship between exposures to nitrates in drinking water (including public and private water supply) and thyroid cancer, hypothyroidism, hyperthyroidism or goitre.
- Studies estimating the risk of thyroid disorders with odds ratio (OR), relative risk (RR); standardised mortality ratio (SMR) and mean differences following exposure to drinking water nitrates.

For some thyroid disorders, there may be no epidemiological studies. However, if any disorders had been demonstrated in animal studies for which there are no epidemiological studies, it is generally reasonable to assume that there is a potential for that same effect to occur in humans

(Brown et al, 1996). Where epidemiological studies exist alongside experimental animal studies for any thyroid disorder, animal studies were used to support the epidemiological evidence.

Following the PRISMA framework, a total of 349 articles were identified through database searches while nine papers were obtained through hand searches of library materials; books, conference papers and from bibliographies of other materials. In all, a total of 358 papers were obtained and after exclusion of duplicates, 326 papers in all were screened. Figure 7 represents the PRISMA flow diagram which explains the exclusion criteria and suggests that papers were excluded if thyroid disorders were not related with nitrate exposure. Also papers were excluded if exposure to nitrates in humans was not related to drinking water.

Figure 7: PRISMA Flow Diagram



Source: Moher et al, (2009)

3.2 THYROID DISORDERS – REVIEW OF ANIMAL STUDIES.

Experimental animal studies (Table 1) suggest that exposure to nitrates in drinking water can disrupt iodine uptake by the thyroid gland resulting in thyroid disorders (Bloomfield et al, 1961; 1962; Alexander & Wolff, 1966; Jahreis et al, 1986). This stems from the ability of iodine inhibition agents, including perchlorate; thiocyanate and nitrate ions to competitively inhibit iodine uptake by the thyroid gland by binding to the sodium – iodide symporter (NIS), a glycoprotein membrane located on the basal side of the thyroid which has been reported in animal studies to result in decreased thyroid hormones (triiodothyronine (T3), thyroxine (T4)) production; hypothyroidism and increased level of thyroid stimulating hormones (TSH) Chronic stimulation of the thyroid gland to produce more hormones by the TSH in the event of low levels of thyroid hormones has been shown to result in thyroid disease; hypertrophy; hyperplasia; adenomas and carcinoma (Hiasa et al, 1991; Capen, 1997 & 1998).

Bloomfield et al (1961 and 1962) reported that exposure to elevated levels of nitrates in drinking water to rats and sheep can competitively inhibit iodine uptake by the thyroid gland and that about 1 per cent of dietary nitrate can inhibit iodine uptake by 30-50 per cent in rats. According to Lee et al (1970), exposure of high concentrations of nitrates to female rats resulted in decreased iodine uptake by the thyroid, hyperplasia, hypertrophy, follicular colloid depletion and increased thyroid weight in the presence of low dietary iodine intake. This suggests that low iodine intake can exacerbate the effect of nitrates on the thyroid gland

Table 1: Summary of Experimental Animal Studies

Author(s)	Animal	Treatment (dose of nitrate or nitrite)	Duration	Outcome
Bloomfield et al, (1961)	Rats and sheep	Diet containing 0.05, 0.1 and 2.5% potassium nitrate (approx.4, 8 and 200 times that of ADI)	30 days	Reduced iodine uptake by the thyroid gland
Bloomfield et al, (1962)	Rat	Two groups of 8 rats exposed to potassium nitrate in dietary.	4 – 5.5 weeks	Reduced iodine uptake in 6-24hrs. Increased thyroid weight
Lee et al, (1970).	Female rats	High-nitrogen-hay diet (40% hay from a plot treated with 450 kg nitrogen/hectare) and a low- nitrogen-hay diet (40% hay from a plot treated with 34 kg nitrogen/ hectare) with three dietary iodine levels: 0.68 ppm (high), 0.23 ppm (medium) and 0.08 ppm (low).	30days	Weight of the thyroid gland, hyperplasia, hypertrophy and follicular colloid depletion in high dose of nitrate low level of iodine.
Kelley et al, (1974)	Mature breeding beagle dogs	Drinking water containing 0, 300, 600 and 1000 ppm sodium nitrate (approx.2.4, 4.8 and 8 times that of ADI)	12days	No change in thyroid morphology. No significant change in either T4 or T3 in any of the groups.
Jahries et al, (1986)	Piglets	Diet containing 3% KNO_3 compared to a free-nitrate diet 6 weeks	5 weeks	Decreased thyroid hormone T4 &T3; decrease weight gain.
Jahreis et al. (1991)	Male and female rats	Diet containing 3% KNO3 (approx.240 times that of ADI)	6 weeks	Decreased food intake by 23-28%, reduced body weight by 35-41%; reduce growth hormone and hypothyroidism
Gatseva et al, (1996)	Male rats	Drinking water containing 50, 100 and 500 mg/l nitrate (~1, 2, and 10 times that of ADI	6 months	Hypertrophy of epithelial cells of thyroid gland follicles in dose of 100 and 500 mg/l
Zaki et al, (2004)	Male rats	Drinking water containing 50, 100, 150, 500 mg/l potassium nitrate (approx.1, 2, 3, 10 times that of ADI)	5 months	Decreased T4 &T3 at dose of 150 & 500 mg/L. Increased weight of the thyroid gland in a dose-dependent manner at doses of 150& 500 mg/l.

Table1 (Continued): Summary of Experimental Animal Studies

Author(s)	Animal	Treatment (dose of nitrate or nitrite)	Duration	Outcome
Tonacchera et al, (2004)	Chinese hamster ovary cells	Comparative iodine uptake inhibition by ClO_4^- ; SCN & NO_3. Potency of ClO_4^- to inhibit iodine uptake was found to be 15, 30 and 240 times that of SCN^-, I^- and NO_3^- respectively	-	Iodine inhibition by nitrates
Eskiocak et al, (2005).	Male rats	Drinking water containing 0, 50, 100, 250, and 500 mg/l sodium nitrate (approx.1.2, 2.4, 6, and 12 times that of ADI)	30 weeks	Reduced iodine uptake. Reduced T4&T4 in dose of 50,250 and 500 mg/l. Increased thyroid gland weight in all doses. Histomorphological changes in the dose of 250 and 500 mg/l.
Mukhopadhy et al, (2005)	Wistar rats	Diet containing 3% KNO3compared with control (~240 times that of ADI) 4 weeks.	4weeks	Increased TSH; reduced T3&T4 and body weight. Increased thyroid weight and increased UIC
Kostogrys et al, (2006)	Male Wistar rats	24 male Wistar rats exposed to $NaNO_3$ in drinking water	3 weeks	Decreased T4; increased TSH. Thyroid hypertrophy and hyperplasia at 300mg/l$NaNO_3$.
Hassan et al, (2008)	Young (3-weeks) and adult (12-weeks) male rats	Drinking water containing 100, 250, and 550 mg/l daily sodium nitrate (2.4, 5.9, and 13 times that of ADI)	4 months	Increased TSH; reduced T3&T4 and reduced body weight. Decreased serum level of protein. Increased urea and creatinine in serum and urine.
El-Wakf et al, (2009)	Male rats	Young and adult male rats exposed to $NaNO_3$ at 100; 150; 550mg/l levels in drinking water.	4 months	Increased TSH; reduced T3&T4 and reduced body weight. Decreased serum level of protein. Increased urea and creatinine in serum and urine.
Hansen et al, (2009)	Pregnant female rats	Examination of endocrine disrupting effects of nitrates on male rat foetuses by exposing pregnant dams to nitrates at dose levels, 17.5; 50; 150; 450 and 900mg/l from gestation day (GD) 7 to GD 21.	21 days	No anti-androgenic effect. No indication of prenatal exposure to nitrates on the thyroid axis of male foetuses

Table1 (Continued): Summary of Experimental Animal Studies

Author(s)	**Animal**	**Treatment (dose of nitrate or nitrite)**	**Duration**	**Outcome**
Oztasan, (2012)	Sprague–Dawley albino rats	Drinking water containing 150 mg/l sodium nitrate (3.5 times that of ADI)	-	Reduced T3 but increased T4
Ciji et al, (2013)	Juvenile fishes (*Labeo rohita*)	Exposure to 2 mg/l nitrite as sodium nitrite	45 days	Decreased T4&T4; decreased body weight
Freitag et al, (2015)	Juvenile Atlantic salmon *Salmosalar*	Drinking water containing 5.2, 10.3 and 101.8 mg/l nitrate.	27 days	No change in levels of T3&T4. No change in body weight
Freitag et al, (2016)	Atlantic salmon embryos	Nitrates in well water 3.76 to 93.15mg/l NO_3-N and thyroid of Atlantic salmon during embryonic development.	-	No effect on the thyroid of embryonic salmon

In a study with nine piglets each in three groups, Jahries et al (1986) fed the first group with 3 per cent potassium nitrate in food and drinking water; the second group diet had but without nitrate and the third group - ad libitum (without nitrate) for 5weeks. The authors reported mean weight of 242; 274; 393g respectively. Decreased T4 and T3 were also reported in the nitrate exposed group compared with the non- exposed group. Jahreis et al (1991) reported decreased growth releasing hormones, thyroidal iodine uptake as well as decreased T3 and T4 concentrations when group of male and female rats were fed a diet containing 3 per cent potassium nitrate for 6 weeks. The exposed rats also experienced reduced food intake by 23-28 per cent; decrease weight gain by 35-41% and hypothyroidism. Similarly, groups of rats exposed to nitrate contaminated water for 90 days reportedly showed hypertrophic changes in the thyroid gland (Knopp et al, 1983 cited by Van Maanen et al, 1994). Two different studies (Horing, 1985; Seffner, 1985 cited by Van Maanen et al, 1994) reported slight increase in thyroid weight;

reduced uptake of ^{131}I (radioiodine) and histological changes in the thyroid gland in rats following exposure of 0.04 - 40mg/l NO_3 for 100 days. Although effects were reported at all dose levels, no evidence of a dose-response relationship was reported. In a study with male wistar rats, Zaki et al (2004) reported that exposure to potassium nitrate (KNO_3) in drinking water for five months resulted in altered thyroid hormone levels, histological modifications and increased thyroid weight. At 150mg/l KNO_3 the decrease in the serum level of thyroid hormone T3 was 34% ($p = <0.05$) and 12% for T4 (the reduction between the thyroid hormones were not statistically significant). At 500mg/l, the decreases in T3 &T4 levels were 44 per cent and 30 per cent respectively ($p< 0.05$). At exposure level of 100,150, 500mg/l, there was a dose dependent increase in thyroid weight (21 per cent, 45 per cent, 75 per cent respectively), $p<0.05$). Thyroid follicle size also increased at exposure level of 150 and 500mg/l. Although the study controlled for iodine, the observed effects according to the authors suggest that exposure to high levels of nitrate in drinking water can impair thyroid functions through the hypothalamus – pituitary – thyroid (HPT) axis.

The effect of chronic exposure to nitrates on thyroid function and morphology was further examined by Eskiocak et al (2005). In this study, five groups of 10 female Wistar rats (3-6months old) were exposed to sodium nitrates ($NaNO_3$) in drinking water at dose levels 0; 50; 100; 250 and 500mg/l over a period of 30 weeks. Decreased radioiodine uptake was reported in the exposure group of 50mg/l but this was not statistically different from the control. However radioiodine uptake increased as nitrate dose increased to 250mg/l ($p<0.05$) and 500mg/l ($p<0.01$). Decreased thyroid hormones T3 ($p<0.01$), T4 ($p<0.05$) and increased TSH levels with evidence of hypothyroidism were observed in the 50, 250 and 500mg/l groups, but not in the 100mg/l group where the total thyroxine level was observed to increase. The weight of the thyroid gland also increased in all the exposure groups compared to the control group. Also

histomorphological changes were observed in the 250 and 500mg/l exposure groups. This study though limited by lack of information on dietary iodine status is consistent with the findings by Zaki et al (2005) that exposure to high levels of nitrates affects thyroid function at the HPT axis. In a study with two groups (eight control, eight treated) of male Albino Sprague Dawley rats exposed to sodium nitrate in drinking water for 8 weeks, Oztasan (2012) reported that exposure to 150mg/l sodium nitrate resulted in increased thyroid hormone T4 and decreased T3 in the treated group ($p<0.05$) , while juvenile fish (*Labeorohita)* exposed to nitrite in drinking water for 45 days showed 84.5 per cent and 94.06 per cent reduction in T_3 and T_4 respectively (Ciji et al, 2013) indicating that exposure to nitrates in drinking water can influence thyroid function.

Tonacchera et al (2004) evaluated the relative potency of the three most important environmental inhibitors of iodine uptake by the thyroid gland (perchlorate (ClO_4^-), thiocyanate (SCN^-) and nitrate (NO_3^-). The evaluation involved exposing Chinese hamster ovary cells to varying concentrations of the three anions separately and in a mixture, and measurement of $^{125}I^-$ (radioiodide) uptake. The ability of ClO_4^- to inhibit iodine uptake was found to be 15, 30 and 240 times that of SCN^-, I^- and NO_3^- respectively. Although this study suggests that nitrate is a less potent iodine inhibitor when compared with ClO_4^- and SCN^-, its effect on the thyroid gland (according to the authors) when present alone or in a mixture cannot be distinguished from the effect of either ClO_4^- or SCN^- . Thus, the effect of nitrates on the thyroid gland which present in a mixture with other iodine inhibitors is additive, with no evidence of synergism or antagonism (Tonacchera et al, 2004). In rats, the effect of nitrates on the thyroid gland was evaluated with exposure to 3 per cent potassium nitrate (KNO_3) in diet for four weeks (Mukhopadhyay et al, 2005). When compared to the control group, the group of rats exposed to nitrates showed increased thyroid gland weight ($p<0.001$), reduced thyroid peroxidise activity ($p<0.01$), decreased serum T4 ($p< 0.01$) and T3 ($p<0.001$) levels. Also increased level of TSH ($p<0.001$)

was reported in the exposed group. Although the exposure duration was short, the development of hypothyroidism and enlarged thyroid gland is an indication of the possible goitrogenic effect of nitrate exposure. In a six-months continuous experiment with 48 viripotent white female rats, line "Vistar", divided into a control group and three experimental groups of 12 rats each, Gatseva et al (1996), reported microscopic changes in the thyroid gland; liver; kidneys; small and large intestines as well as the stomach of the animals that received drinking water containing 50, 100 and 500 mg/dm^3 of nitrates compared to the control group that received 7mg/dm^3 nitrate in drinking water.

Kostogrys et al (2006) reported decreased urinary iodine with increasing nitrate intake in a study in which 24 male Wistar rats were exposed to sodium nitrate in drinking water for three weeks. Despite the short exposure duration and the small number of animals in the study, decreased serum T4 hormone, increased serum TSH, thyroid follicular cell hyperplasia and hypertrophy were observed at the highest exposure level of 300mg/l. This study, according to the authors, demonstrates that nitrates can act as a goitrogen by inhibiting iodine uptake by the thyroid gland, affecting the thyroid-pituitary hormonal axis in a similar way to iodine deficiency. Also, Hassan et al, (2008) and El-Wakf et al (2009) reported a dose dependent decrease in thyroid hormone T3 and T4 when male rats aged 3 and 12 weeks were exposed to sodium nitrate in drinking water at varying concentrations (100; 150; 550mg/l). Increased level of TSH and consequent increase in the weight of the thyroid gland was also observed, indicating hypothyroidism. Reduced body weight was observed in the youngest animals (three weeks old) at all levels of sodium nitrate exposure while reduced weight gain was only observed in the older animals at the highest nitrate exposure level (550mg/l). According to the authors, the reduced weight gain may be attributed to the low serum total protein and protein fractions (albumin and globulin), as well as high level of urea and creatinine found in both serum and urine of all the exposure animals. This study

although limited by the short duration of exposure suggests that prolonged exposure to high levels of nitrate in drinking water can impair thyroid function in both young and older animals but the very young are generally more susceptible to the adverse effects of nitrate exposure.

Although these studies have reported some form of thyroid dysfunction as a result of nitrate exposure, some studies have also reported negative findings. Kelly et al (1974) reported no difference in thyroid function measured by T3 and T4 levels in adult beagle dogs after receiving sodium nitrate in drinking water at 0, 300, 600 or 1000 mg/L for one year or in any puppies from the dams receiving the above doses. Hansen et al (2009) examined the endocrine-disrupting effects of nitrates on male rat foetuses by exposing pregnant dams to nitrates at dose levels, 17.5; 50; 150; 450 and 900mg/l from gestation day (GD) 7 to GD 21. At GD21, foetuses were examined for anogenital distance, plasma thyroxine levels, testicular and plasma levels of testosterone and progesterone, testicular testosterone production and histopathology and endocrine disrupting effects. No anti-androgenic effect was reported. Also, there was no indication that prenatal exposure to nitrates affected the thyroid axis of male foetuses. However, the short duration of the study (GD 7-21) may have accounted for the result. Also, no significant effect on total serum levels of T3 and T4 was observed in 27 days exposure of Atlantic salmon to water containing 5.2, 10.3 or 101.8mg/l nitrate (Freitag et al, 2015). Similarly, Freitag et al (2016) reported that nitrates in drinking water did not affect the thyroid of Atlantic salmon during embryonic development. Nitrate in well water was 3.76-93.15mg/l NO_3-N.

Although the majority of animal studies (Bloomfield et al, 1961; 1962; Gatseva et al, 1996; Zaki et al, 2004; Tonacchera et al, 2004; Mukhopadhyay et al, 2005; Eskiocak et al, 2005; Kostogrys et al, 2006; Hassan et al (2008); El-Wakf et al, 2009; Oztasan, 2012; Ciji et al, 2013) have reported thyroid disorders including hypothyroidism; decreased serum thyroid hormones (T3 and T4), thyroid peroxidase activity, increased levels of TSH; increased thyroid weight or volume,

thyroid hypertrophy and hyperplasia as a result of exposure to nitrates in drinking water, the majority of the effects occurred at high nitrate concentrations. However, they demonstrate the role of nitrate exposure in altering thyroid functions through the HPT axis.

3.3 THYROID DISORDERS - REVIEW OF EPIDEMIOLOGICAL STUDIES

Epidemiological studies have also suggested that exposure to nitrates in drinking water may be related to thyroid disorders. This review updated review by IARC (2010); Health Canada (2013) and Bahadoran et al, (2015).

A summary of the studies by author(s), year of study, study location and design as well as key findings is presented in Table 2.

Table 2: Summary of Epidemiological Studies

Author(s); year of study; location & study design	Endpoint	Nitrate levels in water (mg/l)	Population	RR/OR (95%Cl)	Comments
Van Maanen et al (1994); Cohort study; Netherlands	Thyroid gland hypertrophy	0.02-131	60 women	p<0.05	Association with thyroid hypertrophy at >50mg/l.
Gatseva et al (1997): Cross -sectional study, Bulgaria	Goitre morbidity (children 3-14yrs)	70-90 20-41 (control)	325 school children	OR = 6.11(3.02-12.5), p<0.05.	Strong association with goitre in both boys & girls
Gatseva et al, (1998): Cross -sectional study; Bulgaria.	Goitre incidence in children (6-14yrs) 1990-1996	63-69 (control) 15-24	359 school children	OR = 2.11(95%Cl: 1.31-3.39), p<0.05.	High incidence of goitre in high nitrate area. Statistically significant difference in goitre incidence btw the exposed & control group.
Vladeva et al, (2000); Comparative analysis; Bulgaria	Comparative analysis of studies on goitre 1995-1998 in two villages	-	School children	OR = 3.97 (2.41-7.03), p<0.05. OR = 1.97 (1.42-2.74), p <0.05	Significant association with goitre. Decreased prevalence btw 1995 &1998
Hampel et al, (2003); Germany	Prevalence of goitre and thyroid nodule.	Nitrate level in urine	3059 healthy adults (18-70 yrs. old)	r = 0.18, p <0.01	No correlation with thyroid size at 55.2mg/nitrate in urine. Weak correlation above 60mg/nitrate in urine
Gatseva & Agriova (2005): Cross-sectional study, Bulgaria	Goitre in school children (11-14yrs)	10-95	177 school children	OR = 8.1 (1.67-39.7) *P = 0.004* OR = 4.61(1.52-13.96)	Strong positive association with iodine deficiency. Strong association with goitre
Tajtakova et al (2006): cohort study, Slovakia.	Increased thyroid vol. in school children (10-13yrs old).	51-274	324 HNA 764 LNA (School children).	P <0.03	Increased frequency of thyroid volume and subclinical hypothyroidism.

Table 2(continued): Summary of Epidemiological Studies

Author(s); year of study; location & study design	**Endpoint**	**Nitrate levels in water (mg/l)**	**Population**	**RR/OR (95%Cl)**	**Comments**
Hunault et al, (2007); Randomised controlled non-inferiority trial; Netherlands	Thyroid function after sub-chronic exposure to nitrates.	15mg/kg sodium nitrate & distilled water	20 (men and women)	^{131} I- uptake 35.% (-0.5-7.3)- exposed 4.8%-1.4-11.0 (control)	No significant anti-thyroid effect of nitrates on humans
Radikova et al 2008.Cohort study, Slovakia	Increased thyroid volume/ disorder	51-274	324 HNA 764 LNA	p<0.03	Increased thyroid volume/subclinical hypothyroidism
Gatseva & Argirova (2008a) Cross-sectional study, Bulgaria	Goitre prevalence in pregnant women and children (3-6yrs)	8-93	26 pregnant women 50 children	(Pregnant women) OR = 5.29 (1.0 - 27.94), p=0.045 Children OR = 2.33 (0.85-6.41) P =0.14	Positive association with goitre with increasing NO_3 level
Gatseva & Argirova (2008b) Cross-sectional study, Bulgaria	Goitre prevalence in school children 7-14yrs	8-75	156	OR = 3.01 (1.29-7.027) P= 0.0105	Positive association with goitre
Below et al,(2008); cross-sectional study; Pomerania; Germany	Influence of nitrate on thyroid volume	Mean urinary nitrate level 53.1mg/l.	3772 men/women (20-79yrs old).	P <0.05 P = 0.67	Difference in thyroid vol. btw low &high urinary nitrate. No statistically significant association with goitre when adjusted for age, sex, BMI & smoking habit.

Table 2(continued): Summary of Epidemiological Studies

Author(s); year of study; location & study design	**Endpoint**	**Nitrate levels in water (mg/l)**	**Population**	**RR/OR (95%Cl)**	**Comments**
Blount et al, (2010); prospective study; USA	Sodium/iodide inhibitors across the placenta	Geometric mean of NO_3 in maternal urine 47900µg/l	150 women	-	No iodine transport inhibition across the placenta by NO_3
Ward et al (2010): Cohort study, Iowa USA	Thyroid cancer incidence.	1.6 3.7 7.6 10.8	21, 977 (women, aged 55-69)	RR = 2.2 (0.83-5.76) p = 0.02 at 10.8mg/lNO_3 RR = 2.6 (1.09-6.19) at ≥22mg/l for ≥5yrs. P=0.040	Increased risk of thyroid cancer with nitrate level of 10.8mg/l NO_3. Association with thyroid cancer at ≥22mg/l for ≥5yrs No association with hyper -or hypothyroidism
Kilfoy et al (2012): Cohort study, Pennsylvania, USA	Thyroid health	<28.6 >28.6	2543 1336 (f) 1207(m)	OR = 0.95 (0.27-3.28) P = 0.45 OR = 0.86 (0.39-1.91) OR = 0.89 (0.52-1.52) OR = 1.60 (1.11-2.32)	No significant association with clinical or subclinical hyperthyroidism in men & women. No association with clinical hypothyroidism in men or women. Slight association with subclinical hypothyroidism in women but not in men.
Drozd et al, (2015); analytical study; Belarus	Thyroid post – Chernobyl paediatrics cancer incidence	40-185	Children <18yrs	P = 0.30 P = 0.004	No association with thyroid cancer. Risk of thyroid cancer by radiation modified by high nitrate exposure.
Mehrnejat et al, (2015); descriptive analytical study; Ishafan; Iran.	667 infants diagnosed Congenital hypothyroidism 2010-2013	29 - 36	275, 485 infants	p = 0.39	No statistically significant association with nitrate

In a cross-sectional study in the Netherlands, Van Maanen et al, (1994) evaluated the effect of consuming nitrate contaminated water on the thyroid gland in a population of 60 females aged 40-53 years who had no disease, did not use medication, were not pregnant and had no outdoor jobs. The women were divided into four groups with two groups exposed to low (0.02mg/l, n= 21) and medium (17.5mg/l, n= 23) concentration of nitrates in tap-water and two groups exposed to medium (22±10mg/l, n = 6) and high (131±76mg/l, n = 10) concentration of nitrate in well-water. The result of the investigation showed that as the rate of consumption of water containing nitrate increased, the concentration of urinary and salivary nitrate also increased. Also, increased thyroid volume (hypertrophy) was reported among the group exposed to high nitrate concentration (>50mg/l) in drinking water compared to the low and the two medium exposure groups. This finding according to the authors suggests that a competitive interaction between nitrates and iodine resulted in decreased iodine uptake by the thyroid and consequent enlargement of the thyroid gland. Although there was no difference in thyroid volume between the low and the medium tap-water exposure groups or between the low and the medium well-water exposure groups, there was a positive correlation between nitrate concentration in drinking water and thyroid volume. However, the thyroid hypertrophy observed was associated with low TSH levels in serum and high thyroxine (T4) levels in the high nitrate (>50mg/l) exposure group compared to the medium exposure groups indicating the occurrence of hyperthyroidism. Although iodine intake in the study groups was optimal, about 43 per cent of the study population showed a moderate iodine deficiency. A moderate iodine deficiency can increase the ability of nitrates in drinking water to competitively inhibit iodine uptake by the thyroid gland even when nitrate concentration in drinking water is <50mg/l (Horing et al, 1988 cited by Van Maanen et al, 1994). Although nitrate concentration in the medium tap-water exposure group was lowered from 32mg/l to 17.5mg/l about 1-5years prior to the commencement of the study which resulted in a lower mean intake of nitrate in drinking water, the mean urinary nitrate

excretion in this group was of the same order of magnitude as before it was lowered. This, according to the authors suggests that the rate of elimination of nitrates from the body is very low after a prolonged exposure to high nitrate concentrations. The finding of thyroid hypertrophy (goitre) in this study is consistent with a previous study in the Netherlands (Horing et al, 1988 cited in Van Maanen et al, 1994) which reported increased goitre cases in a group of 12-15 year old females on an iodine deficient diet exposed to 22.5mg/l nitrate in drinking water compared to a group exposed to 7.5mg/l. This study is however limited by the fact that there was no indication whether other iodine inhibitors such perchlorate and thiocyanate which can have similar effect on the thyroid gland were controlled in the study thereby making it difficult to conclude that the effect reported was due to nitrates alone. However, even when present in a mixture with other iodine inhibitors, nitrates can competitively (in a dose additive fashion) inhibit iodine uptake by the thyroid gland with no evidence of synergism or antagonism (Tonacchera et al, 2004).

In Bulgaria, Gatseva et al (1997) reported an investigation of goitre morbidity in 1995 among 177children 3 -14 years (98boys; 79girls) from the village of Karadzhalov in the district of Plovdiv. Nitrate concentration in drinking water was 70-90mg/l between 1990 -1994. Goitre data was compared with that of 148 children (77 boys; 71 girls) of the same age group from the village of Tatarevo (control) in the same district with nitrate concentration of 20-41mg/l in drinking water. In all, 325 children were involved in the study and the two villages had been included in the anti - goitre (iodine) prophylaxis programme in the country prior to the study. Information on nitrate concentration in drinking water was obtained from the district's water Institute of Hygiene and Epidemiology. The result of the investigation showed a statistically significant association with goitre in children living in the high nitrate level village (62/177) compared to children (12/148) living in the control village, OR = 6.11(95%Cl: 3.02-12.5),

$p<0.05$. Among boys, the association with goitre (28/98) was statistically significant in the high nitrate village ($p<0.05$) when compared with boys (0/77) in the control village. Statistical significant association was also reported in girls (34/79) in the high nitrate village compared with 12/71 in the control village, OR = 3.7 (95%Cl: 1.63-8.59), $p<0.05$). When this study was repeated in 1998 with 136 children from the experimental village and 93 children from the same control village, the association with goitre in the children was not statistically significant, OR = 2.06 (95%Cl: 0.89-4.84), $p>0.05$. In a comparative analysis of the 1995 and 1998 studies, (Vladeva et al, 2000) reported that although there was a decrease in the incidence of goitre in 1998 i.e. reduced OR from 6.11 in 1995 to 2.06 in 1998, the association with goitre in the children aged 3-14years in the two studies between 1995 and 1998 (when summed up) was statistically significant, OR = 3.97 (95%Cl: 2.41-7.03), $p<0.05$. Among boys, the OR decreased to 2.25 in 1998 and in the exposed girls it decreased from 3.71 in 1995 to 2.02 in 1998. However, when the OR in the two studies were summed up, the association with goitre in the boys was statistically significant, OR = 8.27(95%Cl: 3.11-32.19), $p< 0.05$, and also statistically significant in the girls, OR = 3.0 (95%Cl: 1.57-5.95), $p<0.05$. This also suggests that the prevalence of goitre was higher in the boys than in the girls.

In a similar study in 1995, Gatseva et al, (1998) investigated the incidence of goitre among 181 children 6-14 years (83 girls and 98 boys) from the village of Ivailo, a region of Pazardjik where nitrate concentrations in drinking water were above the WHO drinking water standard (range 64-69mg/l) during 1990 -1994. The goitre data was compared with those of 178 children (91girls & 87 boys) of the same age group from the control village of Mokrishte in the same region where nitrate concentration in drinking water in the same period, 1990-1994 was 15-24mg/l. In all, thyroid status of a total of 359 children was examined in the study. The result of the investigation showed that 74/181or 40.9% (38 girls and 36 boys) of children who lived in the high nitrate

exposure village had goitre compared to 44/178 or 24.7% (30 girls and 14 boys) of the children who lived in the control village. The association with goitre was statistically significant, OR = 2.11(95%Cl: 1.31-3.39), $p<0.05$. The association was statistically significant in the boys, OR = 3.3 (95%Cl: 1.42-6.52), $p<0.05$ but not significant in the girls, OR = 1.72 (95%Cl: 0.89-3.33), $p>0.05$. Although iodine content of drinking water in both villages was low (0.9mg/dm^3 in Ivailo and 1.0mg/dm^3 in Mokrishte), other drinking water contaminants that may be associated with goitre were also low and within the drinking water standard, suggesting a role for nitrates in goitrogenesis. When this study was repeated in 1998 but this time with 444 children (252 from Ivailo and 192 from Mokrishte), the association with goitre was still statistically significant, OR = 1.84 (95%Cl: 1.15-2.96). Across the genders, the association was neither statistically significant in the boys, OR = 1.90 (95%Cl: 0.99-2.73), $p>0.05$ nor in the girls, OR = 1.85 (95%Cl: 0.94-3.67), $p>0.05$.

A comparative analysis of the 1995 and 1998 studies, (Vladeva et al, 2000) suggests that, although there was a decrease in the prevalence of goitre in the villages in 1998 i.e. a decreased OR from 2.11 in 1995 to 1.84 in 1998, the association with goitre amongst the children in the two studies between 1995 and 1998 (when summed up) was statistically significant, OR = 1.97 (95%Cl; 1.42-2.74), $p<0.05$. In boys, whilst there was a decreased in OR from 3.03 in 1995 to 1.90 in 1998, the association with goitre in the two studies was statistically significant when summed up, OR = 2.34 (95%Cl: 1.43-3.89), $p<0.05$. Although there was no statistically significant difference in goitre in the girls (OR = 1.72 in 1995 and 1.85 in 1998), the association with goitre in the two studies was statistically significant when summed up, OR = 1.78 (95%Cl: 1.12-2.84), $p<0.05$. This study, as in the first pair of villages also suggests that the prevalence of goitre was higher in boys than in the girls. The reason for the decreased goitre trend between 1995 and 1998 in the two pairs of villages as noted by the authors was due to improved iodine

prophylaxis in the villages between 1995 and 1998. According to the authors, increased iodine prophylaxis from the weekly recommended dose to 1mg for children 3-10years and 2mg for children 11-14 years can decrease the ability of nitrates in inhibiting iodine uptake by the thyroid gland and influence goitre prevalence in a population.

Also in Bulgaria, Gatseva & Argirova (2005) in a cross-sectional study investigated the iodine status of 63 school children (aged 11-14) in the villages of Chenogorovo and 114 school children of same age in the village of Parvenez. Nitrate concentration in drinking during the participants' childhood (1983-2005) was 95mg/l in Chenogorovo and 10mg/l in the control village, Parvenez. The result of the investigation showed that exposure to nitrates in drinking water was significantly associated with iodine deficiency in the children from the village with the highest nitrate level in drinking water, compared to the group living in the low nitrate level area, OR = 8.15 (95% Cl: 1.67 – 39.67), p = 0.004. The association was statistically significant among exposed girls, OR = 9.26 (95% Cl: 1.06-80.25), p = 0.04, but not statistically significant among the exposed boys, OR = 5.8 (95%Cl: 0.49 - 67.50), p = 0.18 due probably to sample size. A clinical examination of the thyroid status also revealed that 17.5 per cent or 11/63 of the children in the high nitrate exposure group had grade goitre (diffused goitre grade 1 with hyperplasia) compared to 4.40 per cent or 5/114 of the children in the control village. This finding also showed that there was a statistically significant difference in goitre between the exposed children and the unexposed children ($p<0.05$). Although, iodine intake was slightly higher in the control village than in the experimental village, iodine status of participants in the two villages was optimum and in accordance with International Council for the Control of Iodine Deficiency Disorders (ICCIDD) criteria (ICCIDD/WHO/UNICEF, 2001; 2007). However, goitre frequency in the exposed group was related with mild iodine deficiency. Iodine deficiency is determined by measuring urinary iodine concentration (UIC) in an individual as follows: severe iodine

deficiency (<20µg/l); moderate iodine deficiency (20-49µg/l); mild iodine deficiency (50-99µg/l); optimum iodine intake (100-199 µg/l); 200-299 µg/l – more than adequate and >300µg/l – excessive iodine intake (ICCIDD/WHO/UNICEF/, 2001; 2007).

Gatseva & Argirova (2008a) investigated the risk of thyroid dysfunction among pregnant women (17-37 years old) and children 3-6 years old from the village of Chenogorovo, with nitrate concentration in drinking water was 93mg/l and the village of Parvenez (control) with nitrate level in drinking water 8mg/l. The number of pregnant women involved in the study was 26 from the high nitrate village and 22 from the low nitrate village (48 pregnant women in total). Although all the women reported using iodised salt in the years preceding the investigation, iodine deficiency was reported in 19.2 per cent or 5/26 of the women from the high nitrate village while 4.5 per cent (1/22) from the low nitrate village had iodine deficiency. There was no statistically significant difference in iodine deficiency between the two groups ($p>0.5$). Also goitre was reported in 9/26 or 34.6 per cent of the pregnant women from the high nitrate village while in the low nitrate village, goitre was reported in 9.09 per cent or (2/22) of the pregnant women. The association with goitre was not statistically significant, OR = 5.3(95%Cl: 1.0-27.94), $p = 0.04$. Whilst the majority of the goitre cases were defused (goitre Grade 1), only one woman from the high nitrate exposure group had goitre Grade II, an indication of moderate iodine deficiency. In the children (50 from the village of Chenogorovo and 49 from Parvenez), iodine deficiency was reported in 22 per cent or 11/50 of the children from the high nitrate village compared to 10.2 per cent or 5/49 from the low nitrate village although all the children were reported to have used iodized salt in the years preceding the investigation. Also goitre was reported in 28 per cent or 14/50 of the children from the high nitrate village while 14.3 per cent or 7/49 was reported from the low nitrate village although the association was not statistically significant, OR = 2.3; (95%Cl: 0.85-6.4), $p = 0.14$. This study according to the authors, suggests

that exposure to high levels of nitrate in drinking water is a risk factor for iodine deficiency and goitre and highlight the need for adequate iodine intake by pregnant women and children who are more vulnerable to the effects of iodine deficiency. The study also found excessive iodised salt intake amongst pregnant women and children and highlights the need for adequate control of iodine prophylaxis in iodine deficient areas and the development of healthy nutrition especially among pregnant women and children who are more vulnerable to excessive iodine intake. Excessive iodine intake is associated with the hyperthyroidism and autoimmune diseases (Delange et al, 1999; Zimmermann et al, 2005).

In another study, Gatseva & Argirova (2008b) examined iodine status and goitre prevalence in 319 school children aged 7-14years (156 school children from the village of Ivailo and 163 school children from the control village of Parvenez) with nitrate levels 75mg/l and 8mg/l respectively. A clinical examination of the thyroid showed that 13.5 per cent or 21/156 of the children from the high exposure village had goitre while 4.9 per cent or 8/163 of the children from the low nitrate village had goitre. The association with goitre was statistically significant, OR = 3.0; (95% Cl: 1.3-7.0), p = 0.015. Although the children from the two villages had used iodised salt in the years preceding the study and iodine status was considered optimum in the population, mild iodine deficiency was recorded in the children from the high nitrate village. Whilst the goitre observed in both groups were diffused (goitre grade I), only two girls from the high exposure village had goitre grade II, an indication of moderate iodine deficiency. As in Gatseva & Argirova (2008a), the study also found excessive iodised salt intake in some of the children which is a risk factor for thyroid diseases. The high nitrate village (Ivailo) was in the same village as in Gatseva et al (1998) but given that there was about a 10 year gap between the studies, this suggests that the population of children involved in the two studies was different. Also the fact that nitrate concentration in Ivialo was between 64-69mg/l in 1995 (Gatseva et al,

1998) and 75mg/l in 2008 (Gatseva & Argirova, 2008b) and between 8-10mg/l in the control village of Parvenez (Gatseva & Argirova, 2005; 2008a&b) suggests that once in groundwater, nitrate pollution can persist for a long time. In groundwater, nitrates can remain for a long time even with when leaching from soil or farmland has stopped (Spalding & Exner 1993; United States Geological Society (USGS), 1999).

The result of the Bulgarian studies are consistent with the finding by Van Maanen et al (1994) that exposure to more than 50mg/l of nitrates in drinking water is associated with iodine deficiency, increased thyroid volume and hypertrophy (goitre). Whilst all the children in the various villages and their families had used iodised salt in the 10 years preceding the study and therefore have optimal nutritional iodine intake, the continued prevalence of goitre in some parts of Bulgaria with elevated concentration of nitrates in drinking water sources suggest that nitrates may be interfering with iodine uptake by the thyroid gland in the population exposed to such water. According to Mukhopadhay et al (2005), the persistence of residual goitre in some countries that have successfully implemented iodine prophylaxis (e.g. salt iodization) may be due to the goitrogenic and anti-thyroidal activities of nitrate exposure.

The Bulgarian studies are strengthened by clinical examination of the thyroid status of participants in each study and evaluation of iodine concentration in urine (ioduria) in accordance with ICCIDD /WHO recommendations and criteria (ICCIDD/WHO/UNICEF, 2001). Possible confounding factors such as perchlorate and thiocyanate exposure were accounted for and ruled out as a possible cause of the iodine deficiency or goitre reported in the population. While perchlorate and thiocyanate have been reported to inhibit iodine uptake by the thyroid gland (Tonacchera et al, 2004; Eskiocak et al, 2005; De Groef et al, 2006), the authors reported that perchlorate is rarely encountered in drinking water in Bulgaria and therefore not routinely

monitored. However, the level of perchlorate in water samples in the study areas was below laboratory detection limit, thus leaving nitrate as a major risk factor for iodine deficiency and goitre in the area. Cigarette smoke and vegetables consumption are major sources of thiocyanate exposure in humans (De Groef et al, 2006) but given that children aged 3-14 years are unlikely to be engaged in smoking this suggests that the prevalence of goitre in the study areas is unlikely to be as a result of thiocyanate exposure. Although there was no information on the smoking habits of any of the pregnant women in Gatseva & Argirova, (2008a), pregnancy puts pressure on the thyroid gland (Aoki et al, 2007), and is known to lead to increased iodine depletion in the thyroid gland (Gatseva & Argirova, 2008b). Although vegetables are a major source of thiocyanate exposure, cooking reduces the bioavailability of thiocyanate in vegetables (Bruce et al, 2004). However, whether present alone, or in a mixture with perchlorate and thiocyanate, nitrates can competitively (additively) inhibit iodine uptake by the thyroid gland with no evidence of synergism or antagonism with the other anions (Tonacchera et al, 2004; Braverman et al, 2005). According to USEPA (1986; 2000b), the effect of chemicals in a mixture with similar mode of action is additive. This view is supported by the International Programme on Chemical Safety (IPCS) (2002); Daston et al (2003); Crofton et al (2005).

The Bulgarian studies are however limited by exposure misclassification bias as nitrate concentrations in drinking water in the study regions were average nitrate concentrations obtained from records of the Regional Inspectorate for Pollution and Public Health Protection. There was no individual exposure assessment. No measurement of nitrate concentrations in tap - water at the place of residence or the amount of tap - water intake by the children or pregnant women as the average nitrate concentrations obtained from the regional water inspectorate was used to infer individual exposure. Also, there was no assessment of the amount of water drank away from home or the use of water from multiple sources e.g. well-water or bottled water. Error

in the measurement of nitrate concentrations in drinking water by the regional water inspectorate (either due to equipment error or human error); use of average nitrate concentrations from public records as proxy for individual exposure and lack of individual exposure assessment can result in non-differential exposure misclassification bias (random error) in the relative risk estimates. Misclassification refers to the classification of an individual, a value or an attribute into a category other than that to which it should be assigned (Hennekens & Buring, 1987). Misclassification of exposure or disease status can be differential or non-differential (Hennekens & Buring, 1987; Kirkwood & Sterne, 2003). While differential misclassification (non-random error) occurs when the proportions of subjects misclassified differ between the study groups; non-differential misclassification (random error) occurs when classification of disease status or exposure occurs equally in all study groups being compared (Hennekens & Buring, 1987; Kirkwood & Sterne, 2003). There are two types of error associated with non-differential exposure misclassification, Classical and Berkson error. While classical error arises when a quantity is measured by some device and one measurement from repeated measurements which vary around the 'true value' is used as a proxy for the average; Berkson error arises when the 'measured value' (group's average) is assigned to individuals in the study group as a 'true value' of exposure in place of individual values (Armstrong, 1998; Heid et al, 2004). The effect of classical error is such that it can bias risk estimates (RR; OR etc.) towards the null (no association) (Zeger et al, 2000; Heid et al, 2004; Goldman et al, 2011). In other words, it attenuates risk estimates (Armstrong, 1998; Goldman et al, 2011), making it most likely that the true effect of exposure is greater than that estimated (Armstrong, 1998). Berkson error on the other hand does not bias risk estimates (Zeger et al, 2000; Goldman et al, 2011) provided the true dose -response is linear (Heid et al, 2004). According to Armstrong (1998), Berkson error does not bias linear regression coefficient, and causes little or no bias in logistic or log linear regression coefficients. However, a typical Berkson error reduces the power of a study

(Armstrong, 1998; Zeger et al, 2000; Heid et al, 2004; Goldman et al, 2011), making it more likely that real associations are not detected (Armstrong, 1998). Given that the Bulgarian studies are ecological in design, they were affected by both Classical and Berkson errors. Misclassification of exposure is unavoidable in ecological studies (Heid et al, 2004) and has long been a major limitation of epidemiological studies of disease and the environment (Zeger et al, 2000). Non-differential misclassification (Classical and Berkson errors) diminishes study power (the chance that a study will find a significant association if one is truly present) (Armstrong, 1998) and this may explain the lack of statistical significance in the reported association between nitrates in drinking water and iodine deficiency in boys (Gatseva & Argirova, 2005) and pregnant women (Gatseva & Argirova, 2008a). While increasing sample size does not reduce misclassification bias, but can increase the study power; correcting for exposure misclassification bias is sometimes possible by having more accurate exposure data (Armstrong, 1998). Despite the bias in relative risk estimates and power loss, the p- values obtained with the usual methods on data subject to non-differential misclassification (random error) are valid (Armstrong, 1998).

In Slovakia, Tajtakova et al (2006); Radikova et al (2008) compared thyroid volume of 324 school children (aged 10-13) from a high nitrate area (HNA) with 51-274mg/l in drinking water and that of 764 school children of same age group from two low nitrate areas (LNA) with nitrate level in drinking water less than 2mg/l. The study reported that among the 10years old children, higher thyroid volume was observed in 28/117 in HNA and 22/236 in the LNA. Among the 13 year olds increased thyroid volume was observed in 96/207 in the HNA and 108/528 in the LNA. Analysis of blood samples collected from the 324 children from the HNA and 100 from the LNA showed no difference in total thyroxine (T4 and T3) levels between the two groups. However, increased levels of TSH and anti-thyroid peroxidase antibodies were observed in 13/324 children

and in 8/324 children respectively in the HNA while no such increase of TSH (0/100) or anti-TPO (0/100) were reported in the LNA. The increased TSH level(>4.0Mu/L) was associated with subclinical hypothyroidism causing the authors to conclude that long term exposure to high nitrate levels in drinking water is associated with increased thyroid volume, increased TSH levels, signs of subclinical hypothyroidism and positive anti – thyroid peroxidase. Although information on possible confounding factors was not provided, the result of these two studies is consistent with the Bulgarian studies in that exposure to nitrates in drinking water is associated with thyroid hypertrophy. As in the Bulgarian studies, these two studies are limited by exposure misclassification bias given that there was no information on amount of tap water drunk.

In Germany, Hampel et al, (2003) examined the correlation between urinary nitrate levels and the prevalence of goitre or nodules (corrected for urinary iodide levels) in 3059 clinically healthy adults (18–70 years; both sexes). Median urinary nitrate level was 55.2 mg nitrate per gram creatinine, (men = 61.5 and women 51.5 mg nitrate per gram creatinine, p = < 0.03) and was not correlated with thyroid size or nodules. However, the authors reported a weak correlation between nitrate level in urine and thyroid size (r = 0.18, P <0.05) in 71 adults with decreased iodide in urine (p = < 50 μg/g creatinine). Further, there was a weak correlation between urinary nitrate concentrations above 60 mg nitrate per gram creatinine in1166 adults and thyroid size (r = 0.18; P < 0.01). Given that this study was published in German, the only English translation of the abstract did not state the source of nitrate exposure (diet, drinking water or both) and if from drinking water, the concentration of nitrates in the water was also not stated.

In a cross-sectional study also in Germany, Below et al (2008) analysed the influence of nitrate exposure from different sources on the thyroid volume of 3772 adults (20–79 years old; 1971 men, and 1802 women) in a previously iodine deficient area. The study measured mean urinary

nitrate concentrations as an estimate of nitrate exposure from various sources and reported mean urinary nitrate concentration of 53mg/L and 69mg/l (75th percentile) indicating a significant dietary nitrate exposure in the entire population. The study result showed a statistically significant difference in thyroid volume between persons with and without high urine nitrate level ($p = <0.05$), but when adjusted for age; sex and possible confounding; no difference in thyroid volume was observed ($p = 0.47$). Although the proportion of participants with goitre in the high urine nitrate (115 ± 2.2 mg/L) group was 35.5 per cent and the proportion in the normal urine nitrate (32 ± 0.2 mg/L) was 34.7 per cent, the association with goitre was not statistically significant ($P = 0.69$) even when adjusted for age; sex; smoking and body mass index. Although nitrate levels in drinking water serving the region were reported as 2.5-10mg/l, the proportion of goitre as a result of nitrates exposure in drinking water was not stated. Although the authors stated that the study population had sufficient iodine intake through iodine supplementation, no measurement of urinary iodine was reported; in addition, no measurements of thyroid hormone levels were made.

In a randomised controlled non-inferiority trial, Hunault et al (2007) reported no significant anti-thyroid effect in humans following exposure of 15 mg/kg sodium nitrate in drinking water for 28 days. This study involved 10 individuals who received 15mg/l sodium nitrate and 10 individuals who received distilled water only. Both groups followed an iodide restricted and low-nitrate diet prior to and during the study period and this was verified through measurement of urinary iodine and plasma nitrate levels. At the end of the study period (day 28) plasma nitrate concentration differed by 27mg/kg between the treated and control groups. At (day 29), no significant effects on thyroidal uptake and thyroid hormone (T3, rT3, T4, TSH) plasma concentration were observed. The study concluded that no significant effects on thyroidal ^{131}I uptake and thyroid hormones plasma concentrations were observed after sub-chronic exposition to 15 mg/kg sodium

nitrate among humans. The study according to the authors was limited by small sample size, short exposure duration and the use of low nitrate dose when compared with dose normally used in clinical studies for potential therapeutic benefits. The author however, suggested future studies with longer exposure period and use of higher doses of nitrates.

Iodine is vital in the growth and development of the unborn child (Blazer et al, 2003) and iodine deficiency during pregnancy can affect the development of important organs in the foetus, especially the brain (Haddow et al, 1999; Blazer et al, 2003). It has been reported that the sodium - iodide symporter (NIS) is present in the placenta (Dohan & Carrasco, 2003) and provides a pathway for iodine to reach the foetus in the womb (Logothetopoulus & Scott 1956 cited in Blount et al, 2009). Some studies have suggested that nitrates, nitrites and NOCs can cross the placenta (Shuval and Gruener 1972; Fan et al 1987; Bruningfann and Kaneene, 1993, Ward et al, 2005) and induce mutations in the foetus (Inui et al, 1979) and this raises the possibility for the disruption of iodine transport to the developing foetus. However, Blount et al (2009) in a study of perinatal exposure to the three most important iodine inhibitors (perchlorate, thiocyanate and nitrate) to mothers and their new babies reported no evidence of iodine transport inhibition across the placenta by nitrates or either of the other two anions.

In a case – control study of a cohort of 21,977 women (aged 55-69) in Iowa, USA, whose source of drinking water is from various sources including PWS (n = 5,436); public supply (n = 16,043) and bottled water (n = 440), Ward et al (2010) reported very little evidence of association between nitrates in PWS and thyroid cancer. The association was not statistically significant, RR = 1.13; (95%Cl: 0.83 - 3.66). The risk of hypothyroidism and /- or hyperthyroidism was reported to be low in the exposed group. Nitrate concentration in PWS (private wells) was

assumed to be ≥50mg/l although no measurements were recorded. Among women who used public water, the risk of thyroid cancer increased with increasing nitrate concentration. At nitrate concentrations of >10.8mg/l (NO_3^-), the risk of thyroid cancer was 2.2 folds higher in the exposed group than in the control, RR = 2.2 (95%Cl: 0.83-5.76), p = 0.02 when compared to women in the lowest exposure level (<1.6mg/l) where there was no association. The risk of hypothyroidism (OR = 0.98; 95%Cl: 0.86-1.11), p = 0.81 and hyperthyroidism (OR = 0.98; 95%Cl: 0.79-1.21), p = 0.88 at nitrate concentrations >10.8mg/l were lower in the exposed group compared to the control group. However, the risk of thyroid cancer increased 2.6 folds among women exposed to >22.5mg/l NO_3^- in public water for ≥5years, RR = 2.6 (95%Cl: 1.1-6.2), p = 0.04. At nitrate concentration >22.5mg/l for >5years, the risk hypothyroidism was a little higher (6%) in the exposed group, than in the control, OR = 1.06 (95%Cl: 0.93-1.20), p = 0.47 while there was no difference in risk for hyperthyroidism between the exposed and the control groups, OR = 1.01 (95%Cl: 0.82-1.26), p = 0.88. The association with thyroid cancer following chronic exposure of nitrates at concentration >22.5mg/l for ≥5years is consistent with the report that chronic exposure to nitrates can result in increased production of TSH which could lead to thyroid hypertrophy, hyperplasia, adenomas and carcinoma (Hiasa et al, 1991; Capen, 1992 & 1997). However, in a study of paediatric cancer in Belarus post the 1986 Chernobyl nuclear accident, Drozd et al (2015) reported that whilst radiation exposure was associated with thyroid cancer in children (p = 0.03), exposure to nitrates in drinking water (particularly well water) was not associated with thyroid cancer (p = 0.30). Nitrate concentration in well water in the two areas studied was 40-185 mg/l. However, simultaneous exposure to radiation and high concentration of nitrate in drinking water was reported to increase the risk of developing thyroid cancer (p = 0.004), suggesting that nitrate can modify the risk of thyroid cancer following radiation exposure. The investigation also reported that concurrent exposure to radiation and nitrates in drinking water did not confound each other. The findings of this study are consistent

with previous findings that nitrate ions when present with other anions with the same mode of action can competitively inhibit iodine uptake by the thyroid with no evidence of synergism or antagonism (Tonacchera et al, 2004; Braverman et al, 2005) and also consistent with the findings that the effect of chemicals in a mixture with similar mode of action is additive (International Programme on Chemical Safety (IPCS), 2002; Daston et al, 2003; Crofton et al, 2005).

A major limitation of the Iowa study according to the authors is lack of individual exposure assessment as average nitrate concentrations in drinking water at the place of residence on diagnosis was used to determine exposure. There was no assessment of the amount of tap - water drunk or of women who used water from multiple sources, for example, bottled water or drank water from outside the home. Use of average nitrate levels in tap-water at the place of residence at the time of cancer diagnosis or mortality to estimate past exposure and lack of individual exposure assessment can lead to non-differential exposure misclassification bias (Berkson error). Non-differential misclassification (Berkson and Classical errors) diminishes study power (the chance that a study will find significant association if one is truly present) (Armstrong, 1998) and this may have been the reason for the loss of statistical power among women exposed to >10.8mg/l (NO_3^-) where the risk of thyroid cancer increased 2.2 folds (RR = 2.2 (95%Cl: 0.83-5.76)), p = 0.02. However, despite the bias in the risk estimates (classical error) and power loss (Berkson error), the p-value obtained in the usual method on data subject to non-differential exposure misclassification bias (random error) is valid (Armstrong, 1998). While increasing the sample size does not reduce the bias in the relative risk, it can increase the study power (Armstrong, 1998). However, the bias was reduced by excluding women who reported cancer at the 1986 baseline; those exposed to water from multiple sources and women who had used their public supply or well for ≤10years. Despite the aforementioned limitations, this study according to the authors is strengthened by the prospective nature of the study design, complete ascertainment of the cancer register in Iowa, low population mobility and information

on potential confounder e.g. smoking. Although other potential water contaminants such as perchlorate and pesticides were not evaluated in the study, a prior survey of water sources in Iowa showed that perchlorate is not common in the area (Weyer et al, 2009).

Kilfoy et al, (2012) evaluated the relationship between nitrates in drinking water and prevalence of thyroid health in the Old Order Amish community in Pennsylvania USA (aged 18 years and above). The study involved 1,336 females and 1,207 males (2,543 people in all). The concentration of nitrates in well water ranged from 1.54 - 72.2mg/l NO_3^-, with a median level of 28.6mg/l NO_3^-. The investigation reported that exposure to nitrates in private wells was associated with increased TSH level and subclinical hypothyroidism in men and women, OR = 1.32; (95 % Cl: 1.0 - 1.75) at nitrate concentration greater than 28.6mg/l. The association with subclinical hypothyroidism was statistically significant only in women, OR = 1.60; (95%Cl: 1.11-2.32), no association was reported in men, OR = 0.98; (95%Cl: 0.63-1.52). There was no statistically significant association with clinical hyperthyroidism in men, OR = 1.85; (95%Cl: 0.17-20.7), no association with clinical hyperthyroidism in women, OR = 0.70; (95%Cl: 0.16-3.15). There was no association with subclinical hyperthyroidism either in men or women, OR = 0.86, (95%Cl: 0.39-1.91). The increased TSH and evidence of subclinical hypothyroidism reported in women in this study is consistent with the increased TSH and subclinical hypothyroidism reported in school children from a high nitrate area in Slovakia (Tajtakova et al, 2006; Radikova et al, 2008). Given that men can consume more water than women (NAS, 2004), the reason for increased TSH and higher prevalence of thyroid disease in women than in men was not explained but pregnancy and obesity can affect the level of TSH in women (Kilfoy et al, 2012) and this suggests that women may be more sensitive to the effects of thyroid iodine inhibitors (Hollowell et al, 2002). This study is strengthened by measurement of the level of TSH in individual serum samples. This method, according to the authors is regarded as a more

accurate and reliable way of measuring thyroid hormone levels and thyroid disease than self-reporting of thyroid disease. However, the study is limited by lack of individual exposure assessment as the source of water to the residence was not determined, neither was the amount of tap-water intake. Although the source of water was assumed to be from private wells at the place of residence as there were no public water supply serving the area, it is possible that there may be residents who may be consuming bottled or filtered water e.g. reverse osmosis. Use of alternative water source according to the authors can result in exposure misclassification bias. Furthermore, lack of information on the residential history of the study population could have an effect on the results. This is because people could change their place of residence and could as a result be exposed to a different water quality. However, it was reported that the Amish community has a very stable residential history especially the men and if women changed their residences, they only move from their family home to their marital home but remained in the same locality (McKnight et al, 1999), and were therefore likely to use water of similar quality.

In a study of congenital hypothyroidism among infants in Ishafan province, Iran, Mehrnejat et al, (2015) reported no statistically significant association between nitrate concentrations in drinking water (29-36mg/l) with congenial hypothyroidism ($p = 0.39$) in some towns in the Province.

3.4 DISCUSSION

Result of epidemiological studies on thyroid disorders as a result of exposure to nitrates in drinking (Van Maanen et al, 1994; Gatseva et al, 1997; 1998 ; Vladeva et al, 2000; Hampel et al, 2003; Gatseva & Argirova 2005; 2008a & b; Tajtakova et al, 2006; Hunault et al, 2007; Radikova et al, 2008; Below et al, 2008; Blount et al, 2009; Ward et al, 2010; Kilfoy et al, 2012; Drozd et al, 2015; Mehrnejat et al, 2015) are conflicting. The conflicting results may be due to

differences in nitrate concentrations in drinking water across the studies; differences in individual exposure levels; differences in exposure to other risk factors of thyroid disorders; individual variability; differences in iodine status in the study population; differences in the definition of low and high exposure concentrations across the studies. However, there is a convergence on moderate – to - mild iodine deficiency (Van Maanen et al, 1994; Gatseva & Argirova 2005; 2008a & b); hypothyroidism (Tajtakova et al, 2006; Radikova et al, 2008; Ward et al, 2010; Kilfoy et al, 2012) and thyroid hypertrophy (goitre) **(**Van Maanen et al, 1994; Gatseva et al, 1997 & 1998; Hampel et al, 2003; Gatseva & Argirova 2005; 2008a & b; Below et al, 2008; Tajtakova et al, 2006; Radikova et al, 2008) due to reproducibility or consistency across these studies.

3.4.1 META - ANALYSIS

In order to further evaluate the relationship between exposure to nitrates in drinking water and thyroid disorders, meta-analysis was conducted. Based on availability of data, the analysis was conducted on the following outcomes: clinical and subclinical hypothyroidism (Tajtakova et al, 2006; Radikova et al, 2008; Ward et al, 2010; Kilfoy et al, 2012); clinical and subclinical hyperthyroidism (Ward et al, 2010; Kilfoy et al, 2012) and goitre (Gatseva et al, 1997 & 1998; Gatseva & Argirova 2005; 2008a & b; Tajtakova et al, 2006; Radikova et al, 2008).

Meta- analysis is the statistical analysis of a collection of individual studies for the purpose of integrating the findings in order to arrive at a conclusion about that body of research (Glass 1976). It is conducted to assess the strength of evidence present about a disease or treatment with the aim of determining whether or not an effect exists as well as whether the effect is positive or negative. The outcome of a meta-analysis provides a more precise estimate of the

effect of a treatment or risk factors of a disease than individual studies contributing to the pooled analysis; answers questions not posed by the individual studies; settles controversies arising from conflicting studies and generates new hypotheses. Also, it identifies sources of variation in responses i.e. examines the heterogeneity between the studies included in the analysis (Haidich, 2010). Heterogeneity refers to the variation in study outcomes between studies (Higgins & Thompson, 2002; Higgins et al, 2003).

The meta-analysis was conducted in 'R' – software (version 3.3.2 of 31 October 2016). 'R' is a free, open-source and powerful statistical environment for data manipulation, calculation and graphical display. RStudio is a user-friendly tool for running analyses using 'R'. Both R and RStudio are free to use and very popular (Kovalchik, 2013). The Fixed effect and random effect models were ran to test for variations across the studies (heterogeneity). The fixed effect model assume that the only source of variation in the observed outcomes is that occurring within the study (the effect occurring in each study is the same) i.e. there are no differences in the underlying study population; no differences in subjects selection criteria and that treatments are applied in the same way (Stangl & Berry, 2003; Haidich, 2010). The random effect model however, assumes that a distribution of effects exist resulting in heterogeneity among study results (Stangl & Berry, 2003; Haidich, 2010). In other words, it assumes that the effect being estimated in the different studies are not identical, but follow some distribution (Deeks et al, 2011).

Given the assumption in the 'fixed effect model' that the effect of interest is the same in all studies is not usually the case; the model is not considered appropriate when heterogeneity exists in the result of studies included in the meta-analysis (Haidich, 2010). Therefore only the 'random

effect model' is presented in this study because of methodological differences across studies; differences in the level of nitrates in each study; individual variability; differences in individual exposure levels and differences in exposure to other risk factors of thyroid disorders. The random effect model is considered more appropriate because of differences that exist in the studies (Haidich, 2010).

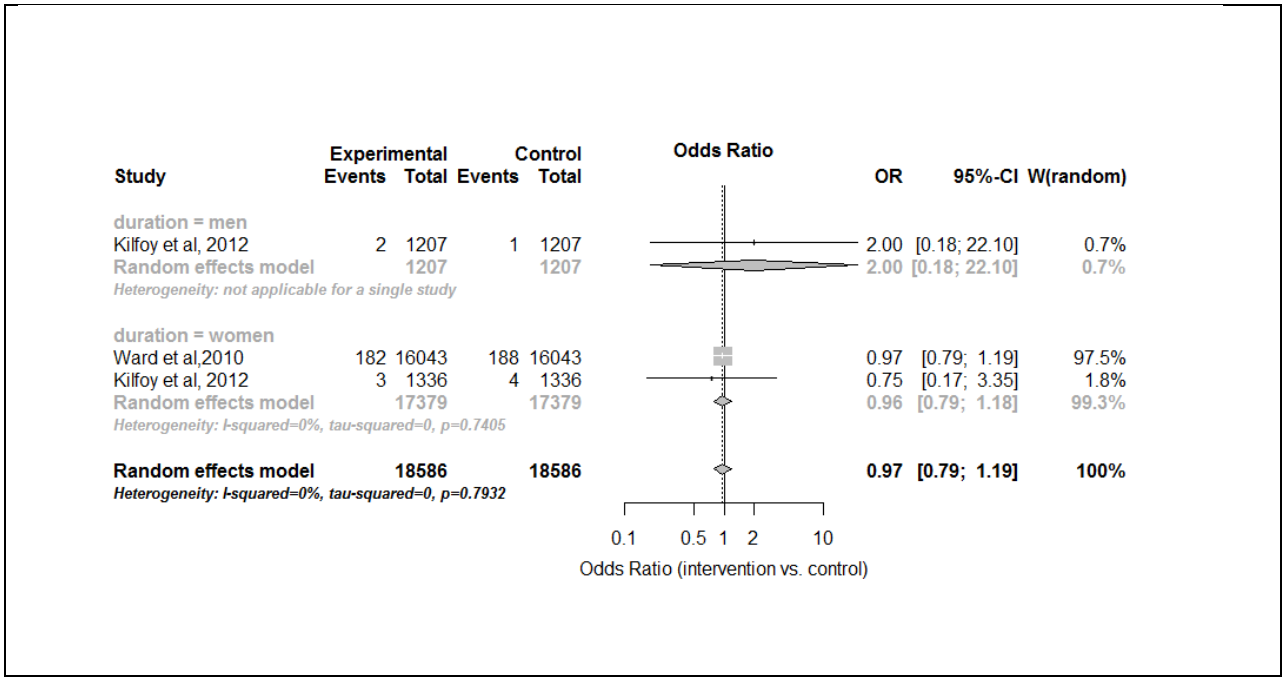
A forest plot shows effect size and precision and is regarded as the most common way to report meta-analysis. Also, it identifies the pattern across effects as well as helping to spot large variations in effect (Kovalchik, 2013). Check for heterogeneity was performed using the I-squared (I^2) in order to quantify the inconsistency across studies and to assess the impact of heterogeneity on the effect estimates. I-squared describes the percentage of variation across studies that are due to heterogeneity rather than chance (Higgins & Thompson 2002; Higgins et al, 2003). According to Higgins & Thompson (2002), the interpretation of I-squared is as follows:

- 0% to 40% - low (might be unimportant).
- 30% to 60% - may represent moderate heterogeneity.
- 50% to 90% - may represent substantial heterogeneity.
- 75% to 100% - considerable heterogeneity.

The p- value in also included in the forest plot to test whether any heterogeneity is statistically significant or not (Higgins et al, 2003). A p- value of 0.10, rather than the conventional level of 0.05, is sometimes used to determine statistical significance. A low p- value ($p<0.10$) provides evidence of heterogeneity of intervention effects or variation in effect estimates beyond chance while a high p-value ($p>0.10$) suggest a no-significant heterogeneity (Higgins et al, 2003).

Figure 8 is the forest plot (random effects model) on clinical hyperthyroidism from two studies (Ward et al, 2010; Kilfoy et al, 2012) and shows an effect estimate, OR = 0.97; (95%Cl: 0.79 - 1.19). This suggest that the risk of this outcome is only 3 per cent (i.e.1- 0.97)*100) lower in the exposed group than the control group and the result is not statistically significant. In other words, there is very little difference in effect between the exposed and non-exposed groups. The analysis also shows no evidence of heterogeneity between the studies, I^2 = 0%; (p = 0.79).

Figure 8: Forest Plot of Clinical Hyperthyroidism (random effects model)



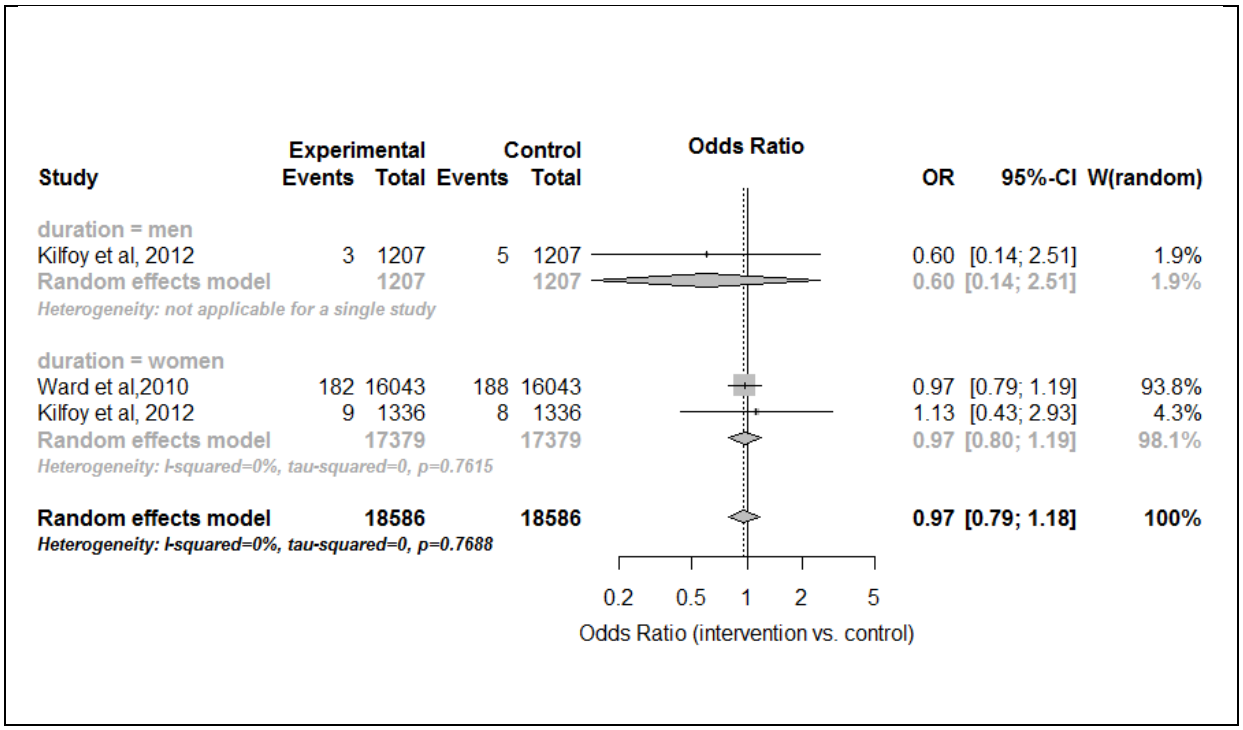
Lack of evidence of heterogeneity is not evidence of homogeneity and may be that the test has low power to detect heterogeneity due to the small number of studies in the analysis (Higgins et al, 2003). While a statistically significant result may indicate a problem of heterogeneity, a non-significant result must not be taken as evidence of no heterogeneity (Higgins et al, 2003). It may be that the test for heterogeneity has low power (when studies have small sample size or when the number of studies are small) to detect heterogeneity when it exist. When there are many

studies in a meta-analysis, the test has high power to detect the smallest amount of heterogeneity (Higgins et al, 2003). The result of this analysis is consistent with that of Bahadoran et al, (2015) which reported that the effect of nitrates in drinking water on clinical hyperthyroidism is lower (2 per cent) in the exposed group than in the control group, OR = 0.98; (95%Cl: 0.79 - 1.21).

SUBCLINICAL HYPERTHYROIDSIM

Figure 9 is the forest plot of subclinical hyperthyroidism which also suggests that the effect of nitrates is 3 per cent lower in the exposed group than the control, OR= 0.97; (95%Cl: 0.79; 1.18). There is no evidence of heterogeneity, I^2 =0%; p = 0.77 due to the low power of the test as a result of the small number of studies in the analysis. More studies are required in order to clarify the relationship between nitrates in drinking water and hyperthyroidism and any heterogeneity between the studies.

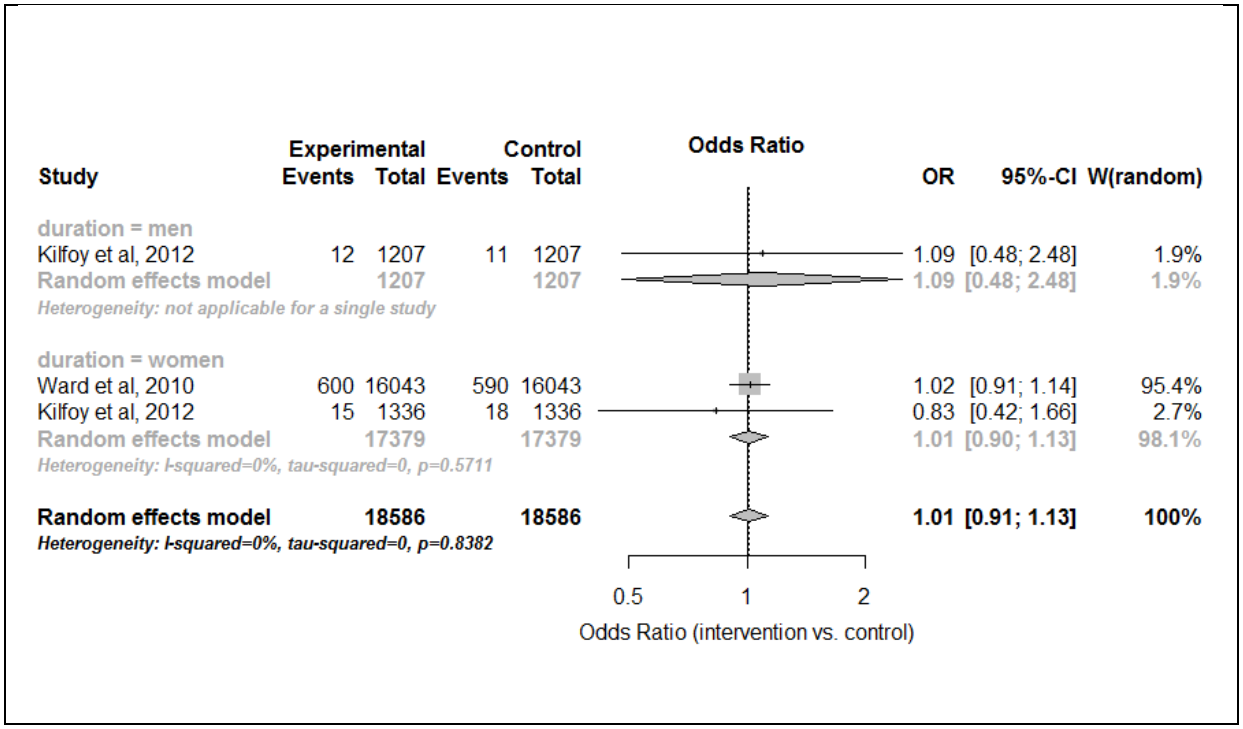
Figure 9: Forest Plot Subclinical Hyperthyroidism



CLINICAL HYPOTHYROIDISM

The forest plot (figure 10) shows that there was no difference in the risk of clinical hypothyroidism between the exposed group (men and women) and the control group and the result is not statistically significant, OR = 1.01; (95%Cl: 0.91-1.13). However, the risk of clinical hypothyroidism is 9% higher in the exposed men (OR = 1.09: (95%Cl: 0.48-2.48) than in women (OR=1.01:95%Cl; 0.91-1.13). There is no evidence of statistically significant heterogeneity (I^2 = 0%, p = 0.88) due to the small number of studies in the analysis. This result is also consistent with the meta-analysis by Bahadoran et al, (2015).

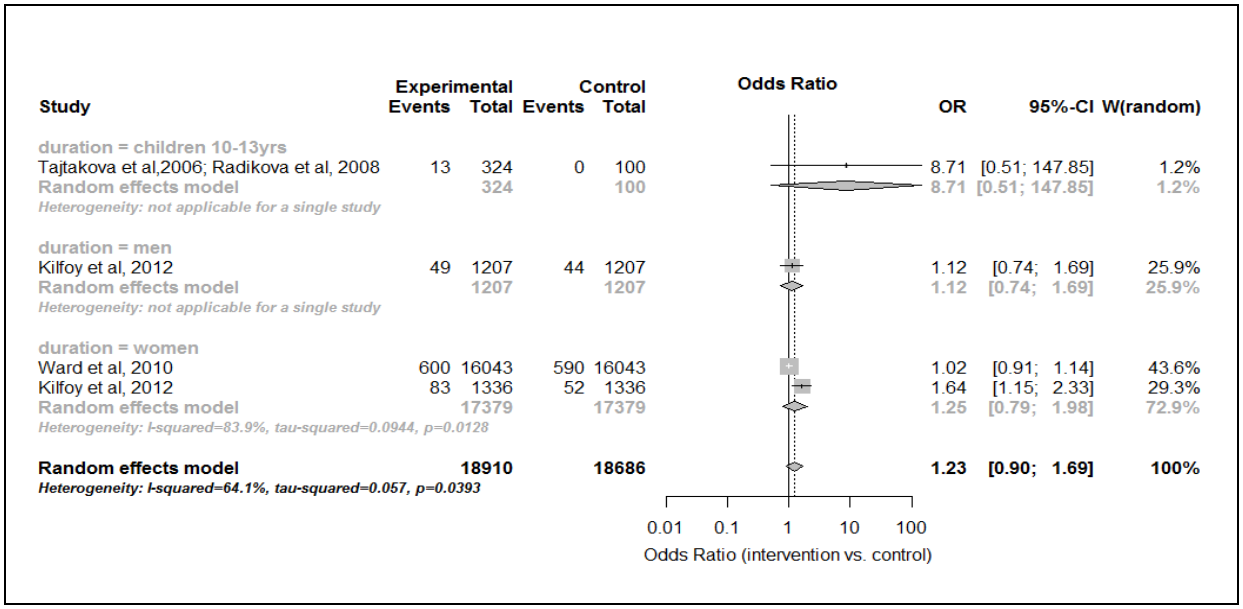
Figure 10: Forest plot for clinical hypothyroidism



SUBCLINICAL HYPOTHYROIDISM

Figure 11 shows the forest plot for subclinical hypothyroidism in the random effect model, OR = 1.23; (95%Cl: 0.90-1.69); which suggests that the risk of this outcome is 23% higher in the exposed group (children, adult men and women) than in the control group.

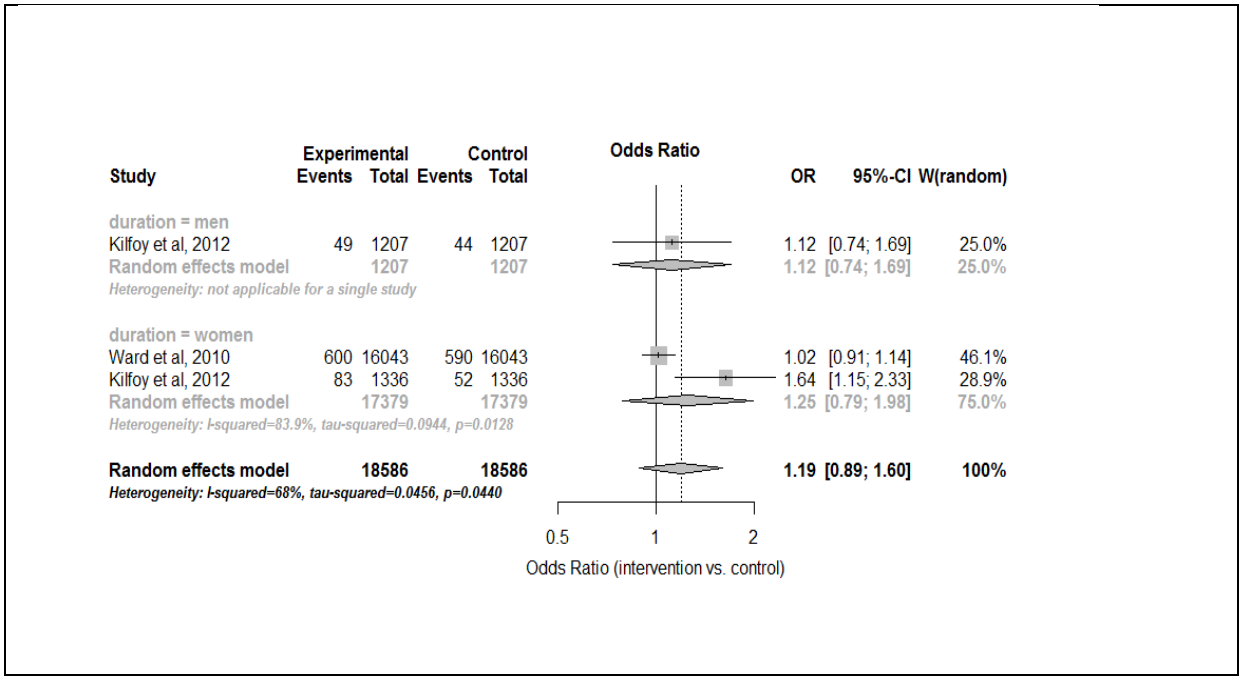
Figure 11: Forest Plot of Subclinical Hypothyroidism



The risk is however 8.7 times higher in children, (OR=8.71:95%Cl: 0.51-147.85) than in adults (men and women) although the result is not statistically significant. Between men and women, the effect of subclinical hypothyroidism was slightly higher in women (OR= 1.25) compared to men (OR =1.12). The large effect in children suggests that children are more susceptible to subclinical hypothyroidism than adults as a result of exposure to nitrates in drinking water. There is substantial statistically significant heterogeneity in the studies (I^2 = 64.1%; p = 0.04) due probably to the inclusion of studies on children and adult males & females in the same analysis. However, when the study on children was excluded in a sensitivity analysis (figure 12), the risk

of subclinical hypothyroidism in the exposed adult males and females was 19% higher in the exposed than in the control group, OR = 1.19; (95%Cl: 0.89 -1.60) and not statistically significant. However, the level of heterogeneity remained substantial and statistically significant, I^2 = 68%; p = 0.04. The substantial heterogeneity may be due to differences in the sample size between the two studies.

Figure 12: Sensitivity Analysis of Subclinical Hypothyroidism



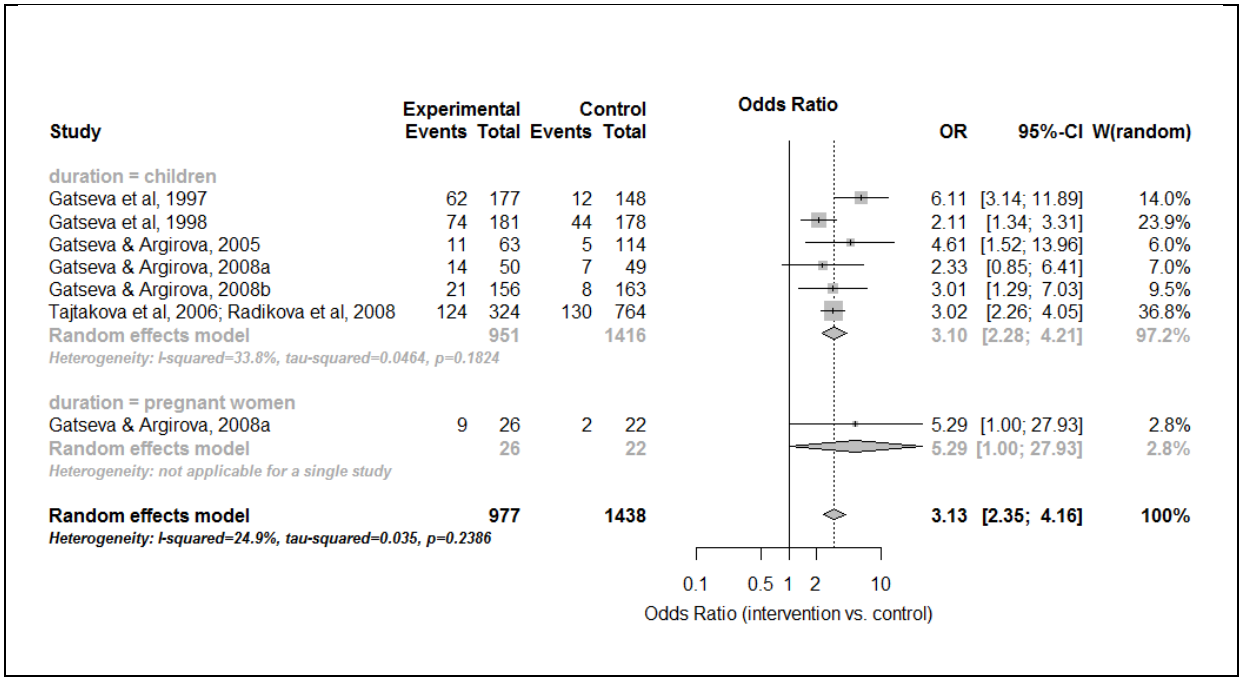
The funnel plot is used to evaluate the potential for bias in selecting studies for meta-analysis given that sometimes large and positive studies or results are published in preference to small or negative studies and it is an important part of any meta- analysis (Haidich, 2010). If publication bias is not present, the plot is expected to have a symmetrical inverted funnel shape with large studies (which tend to have lower standard error) clustering closer to the effect estimate and smaller studies (with higher standard error) scattered on both sides of the larger studies (Light &

Pillemer, 1984; Haidich, 2010). However, due to the small number of studies, funnel plots may not provide any useful information (Haidich, 2010) but are however presented Appendix 2.

THYROID HYPERTROPHY (GOITRE)

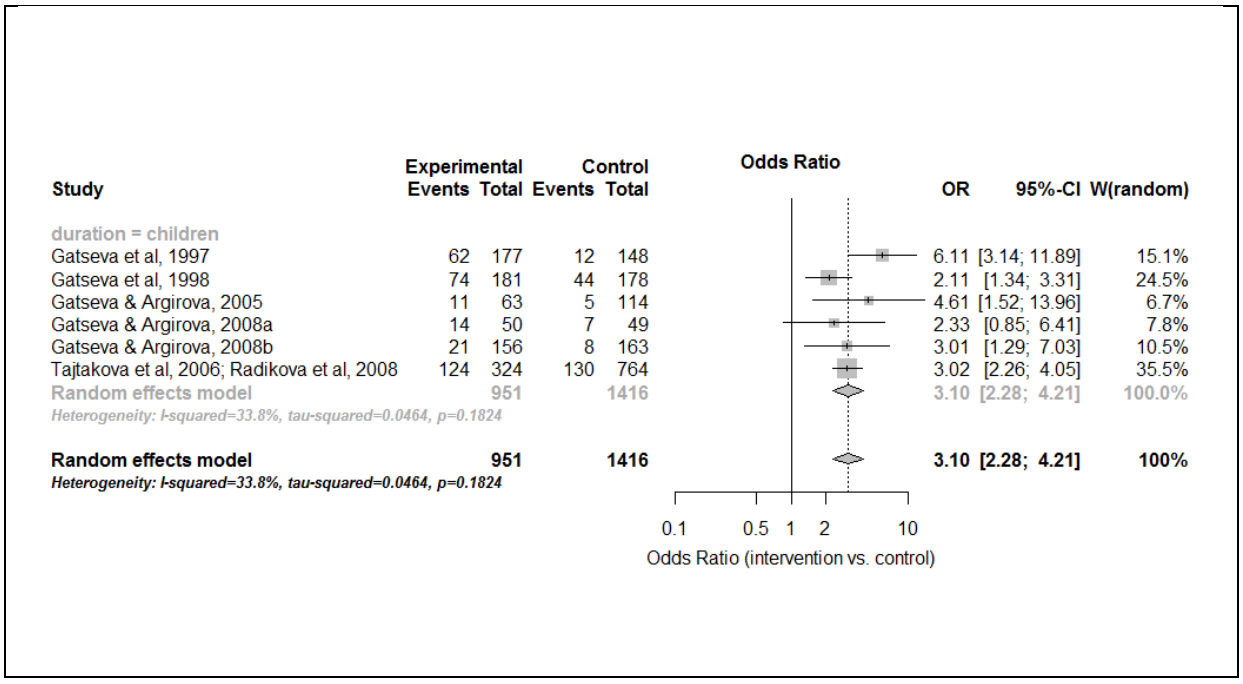
Figure 13 shows the forest plot (random effect model) of subgroups from seven studies for goitre (Gatseva et al, 1997 & 1998; Gatseva & Argirova 2005; 2008a & b; Tajtakova et al, 2006; Radikova et al, 2008). The result shows that the risk of goitre is, OR = 3.13; (95%Cl: 2.35 - 4.16). This suggests that the risk of goitre is 3.13 times higher in the exposed group than in the control group, the result is statistically significant. The test for heterogeneity (I^2 = 25%; p = 0.24) suggests that heterogeneity across the studies is low (<30%) and not significant in accordance with the Higgins & Thompson (2002) interpretation.

Figure 13: Goitre forest plot



The analysis on thyroid hypertrophy (goitre) included studies on pregnant women and children in the same analysis. However, in a sensitivity analysis which excluded pregnant women from the analysis (figure 14), the effect estimates was, OR = 3.10; (95%Cl: 2.28 - 4.21) and also statistically significant. This suggests that the risk of goitre is 3.10 times higher in the exposed children than the control group. Heterogeneity (I^2 = 33.8%; p = 0.18) is low and not significant. The marginal difference (0.03 or 3 per cent) in the effect estimates between the analysis which included pregnant women and the one that excluded pregnant women suggests that children and pregnant women can be analysed together. The large relative risk (odds ratio) in children (OR = 6.11 & 4.61) and pregnant women (OR = 5.29) suggests that the risk of goitre is higher in children, (especially infants) and pregnant women.

Figure 14: Sensitivity Analysis on Goitre



Due to insufficient data, meta- analysis on iodine deficiency and thyroid cancer as a result of exposure to nitrates in drinking water could not be conducted. However, Bahadoran et al (2015) conducted a meta- analysis on thyroid cancer following exposure to nitrates from diet (including drinking water) and reported that the risk of thyroid cancer was 36% higher in the exposed group than in the control groups, RR= 1.36; (95%Cl: 0.67-2.75). The association was however not statistically significant. Although the result of meta-analysis on clinical hyperthyroidism and clinical hypothyroidism are consistent with that of Bahadoran et al (2015), they were unable to conduct a meta- analysis on thyroid hypertrophy (goitre) citing lack of data. This may be due to the ecological nature of available studies on goitre. Although this kind of studies are prone to bias; chance and confounding factors (IARC, 2010), the IARC has relied on them in its classification of arsenic in drinking water as a carcinogen (IARC, 2004).

3.4.2 WEIGHT OF THE EVIDENCE (WoE) ASSESSMENT

Result of meta- analysis suggest that the effect estimates following exposure to nitrates in drinking water is strongest for thyroid hypertrophy (goitre) (OR = 3.13); weak for subclinical hypothyroidism (OR = 1.23) and weakest for clinical hypothyroidism (OR = 1.01) and hyperthyroidism (clinical or subclinical, OR = 0.97). Although causality has not been established between any of these outcomes and exposure to nitrates in drinking water, the Hill Criteria can be used to assess whether the association is causal (WHO, 1999). The Hill criteria (Hill 1965) as enumerated in Box 1 are widely used and still remain the best available criteria for causal inference (WHO, 1999; Swaen & van Amelsvoort, 2009).

The strength of the association i.e. the large relative risk on goitre (expressed as odds ratio) (OR = 3.13) following meta-analysis and the statistically significant association suggests that the

association is unlikely to be due to chance. Although a statistically significant association does not rule out chance as a possible reason for the association, such an explanation is however, unlikely (Hennekens & Buring, 1987).

BOX 1 HILL CRITERIA ON CAUSAL INFERENCE

1. Strength of the association - the stronger the association the more likely that the association is causal.
2. Consistency - if more studies find similar results, the more likely it is that the association is causal.
3. Specificity - a specific exposure should exert a specific effect (there are causal associations that are not specific).
4. Temporality - the causal exposure should precede the caused disease in time.
5. Biological gradient or dose-response - If a dose- response is seen, it is more likely that the association is causal.
6. Plausibility - depends on the current knowledge of the aetiology of the disease. For instance, is it known that the agent or metabolite reaches the target organ, are studies in animal models positive?
7. Coherence - refers to other observed biological effects possibly relevant in the aetiological pathway that make a causal association more likely, for example, histological changes in the target organ.
8. Experimental evidence - if the disease rates go down after the causal agent has been eliminated, it is support for a causal association.
9. Analogy - if a similar agent exerts similar effects, it is more likely that the association is causal.

Source: Swaen & van Amelsvoort (2009).

Whilst the majority of the studies, especially the Bulgarian studies ruled out possible confounding factors e.g. perchlorate and thiocyanate as a reason for the association, evidence of dose-response relationship reported in the studies suggest that exposure to nitrates in drinking water is a risk factor for goitre. The large relative risk (odds ratio) especially in children (including infants) and pregnant women further suggests that the effect of nitrates in drinking water is more profound in children and pregnant women living in iodine deficient areas. This finding is consistent with the WHO epidemiological review (Health Canada, 2013) which reported that the anti-thyroid effect of nitrates is mostly related to exposure in drinking water rather than diet. The effect is however, more profound if there is iodine deficiency but weak if there is adequate nutritional iodine intake i.e. determined by urinary iodine excretion of 150-300µg/day (Health Canada, 2013). The goitrogenic effect of nitrates is more pronounced when there is insufficient dietary iodine intake (van Maanen et al, 1994; Tonacchera et al, 2004). According to van Maanen et al (1994) and Tonacchera et al (2004), chronic exposure to nitrates in drinking water below the drinking water standard of 50mg/l can result in the enlargement of the thyroid gland and goitre if there is insufficient dietary iodine intake. Also, even under normal dietary iodine intake, exposure to elevated levels of nitrates (≥50mg/l) in drinking water can result in decreased iodine uptake by the thyroid gland, resulting in thyroid hypertrophy (goitre) and hyperplasia (van Maanen et al, 1994; Tonacchera et al, 2004; Tajtakova et al, 2006).

Although meta-analysis on iodine deficiency has not been possible due to insufficient data, the evidence of iodine deficiency in children (Gatseva & Argirova, 2008a&b); pregnant women (Gatseva & Argirova, 2008a) and women aged 40-53years (Van Maanen et al, 1994), large relative risk (odds ratio) in children (OR = 8.15) (Gatseva & Argirova, 2005) and the evidence of dose-response in all the studies suggest that children and pregnant women are vulnerable to iodine inhibition as a result of exposure to nitrates in drinking water. Infants are more sensitive

to iodine inhibitors than adults and therefore require more iodine intake in order to produce more thyroid hormones during shortages (Health Canada, 2013). This is because of the short half-life of thyroid hormones in infants (approximately 3 days) (Vulsma et al, 1989) and their inability to store thyroid hormones for more than a day for use during shortages like in adults (Zoeller & Crofton 2005).

Although the effect estimates for hypothyroidism (clinical or subclinical) is weak, the large relative risk (odds ratio) for subclinical hypothyroidism in children (OR = 8.71) in Tajtakova et al, (2006) and Radikova et al, (2008) suggests that children especially infants are more sensitive to this outcome than adults. Although the number of studies on hypothyroidism (clinical or subclinical) is too few to enable a firm conclusion to be drawn, the evidence is supported by result of animal studies (Hassan et al, 2008; El-Wakf et al, 2009). Subclinical hypothyroidism especially during critical or sensitive periods of development can result in impaired foetal development, reduced heart rate and body heat producing capabilities and reduced intelligent quotient (IQ) (Howdeshell, 2002; Kirk, 2006). High TSH is a biomarker for hypothyroidism (De vito et al, 1999; Zoeller et al, 2007). Although hypothyroidism is very common during pregnancy because pregnancy puts pressure on the thyroid gland (Aoki et al, 2007), epidemiological evidence for subclinical hypothyroidism and thyroid hypertrophy (goitre) is biologically plausible and supported by result of animal studies (Wyngaarden et al, 1953; Bloomfield et al, 1960; Gatseva et al, 1996; Zaki et al, 2004; Tonacchera et al, 2004; Mukhopadhyay et al, 2005; Eskiocak et al, 2005; Kostogrys et al, 2006; El-Wakf et al, 2009; Oztasan, 2012; Ciji et al, 2013). Although goitre reported by Van Maanen et al, (1994) was associated with hyperthyroidism (low serum TSH), this has not been supported by another study.

The evidence for hypothyroidism (subclinical) and thyroid hypertrophy (goitre) as a result of exposure to nitrates in drinking water is consistent with the mechanism of action of nitrates on the thyroid gland. The mechanism stems from the ability of nitrate and /-or its metabolite, nitric oxide to inhibit iodine uptake by the thyroid gland resulting in iodine deficiency; decreased thyroid hormone (T3 & T4) production and increased level of serum TSH (Alexander & Wolff, 1966; Greer et al, 2002). Chronic stimulation of the thyroid gland by TSH to produce more hormones in the event of low serum thyroid hormones has been reported in animal studies to result in thyroid diseases including; thyroid hypertrophy; hyperplasia; adenomas and carcinoma (Hiasa et al, 1991; Capen, 1997 & 1998).

Although only two epidemiological studies (Ward et al, 2010; Drozd et al, 2015) were found to have evaluated the relationship between exposure to nitrates in drinking water and thyroid cancer but with conflicting results, Bahadoran et al (2015) conducted a meta-analysis on thyroid cancer following exposure to nitrates from diet (including drinking water) and reported a weak, and non- statistically significant association, RR= 1.36; (95%Cl: 0.67-2.75). The potential for thyroid cancer is biologically plausible given that chronic stimulation of the thyroid gland by TSH to produce more thyroid hormones during thyroid hormones deficiency can result in thyroid hypertrophy (goitre), hyperplasia, adenomas and carcinomas in animal studies (Hiasa et al, 1991; Capen, 1992; 1997). Although thyroid hormones deficiencies can play a role in thyroid tumour production in animals (Kanno et al, 1990; Hiasa et al, 1991; Capen, 1992 & 1997; Crofton 2008) and humans (Crofton 2008; Health Canada 2013) , the role of thyroid hormones deficiencies in thyroid cancer aetiology in humans is not well defined (Schnieder & Brenner, 2003; Croften, 2008). While decreased thyroid hormones (T4&T3) can result in a number of adverse effects, including thyroid tumour and birth defects, it is not clear if humans can get thyroid carcinoma in the same way as rodents because humans are less susceptible to the effect of TSH on thyroid

cells (Croften, 2008), although there is the potential for any adverse effect in animals to also occur in humans (Brown et al, 1996). Nitrates can however, undergo endogenous nitrosation endogenous with amines and amides in food to form N-nitroso compounds (Walker 1990; Bruning-Fann & Kaneene, 1993). The majority of N- nitroso compounds (NOCs) have been reported to induce cancer in animals, including fish, reptiles and five species of primate (Montesano and Bartsch, 1976; Gangolli et al., 1994; Brown, 1999; Vermeer and Van Maanen, 2001). In humans, NOCs is reported to be associated with cancer in the stomach, liver, kidney, oesophagus, oral and nasal cavities, lung, trachea, urinary bladder, pancreas, thyroid and the nervous system (Mirvish, 1991&1995 NRC 1981; Bogovski & Bogovski 1981; Twort et al, 2000; ASTDR, 2001; Weyer, 2003; IARC, 2010). Although it is difficult to prove causality firmly from epidemiological studies due to the observational nature of these type of studies (Wakefield & McElvenny, 2007; Swaen & van Amelsvoort, 2009), however, the strength of the association between nitrates in drinking water and goitre; the consistency of the study results in animal and human studies; evidence of dose-response; biological plausibility and coherence suggests that nitrates in drinking water is a risk factor for goitre. Although all the nine criteria are important, the most important ones included, strength of the association; consistency of results; dose-response; biological plausibility (USEPA, 2005; Wakefield & McElvenny, 2007) and coherence (USEPA, 2005). Although causality has not been firmly established, this finding can be used by policy makers to formulate public health policies or direct intervention programmes in order to protect the public from the anti-thyroidal effects of nitrates in drinking water.

The Hill criteria are not only used in the field of epidemiology, but also in the field of toxicology (Guzelian et al, 2005) and have been adopted by the International Agency for Research on Cancer (IARC) in the evaluation of the carcinogenicity of various chemicals and substances (Swaen & van Amelsvoort, 2009), including nitrates (IARC, 2010). Also, they have been

adopted by the USEPA and Health Canada in the weight of evidence assessment of various chemicals and substances (USEPA, 2005, Health Canada, 1994). Although the Hill criteria are widely used, some authors (Lanes & Poole, 1984; Kundi, 2006) have criticised over - reliance on these set of criteria in assessing the weight of evidence for association between exposure and disease. According to Kundi (2006), over-reliance on these set of criteria could result in ignoring public health intervention programmes on the argument that the available evidence does not fulfil the criteria and proposed that assessment of causal inference should be based on prior knowledge from epidemiological, animal and in- vitro studies. This cautious note from Kundi (2006) is consistent with the view earlier expressed by Hill (1965) and as cited by Phillips & Goodman, (2004) and Rothman & Greenland, (2005), that, there are no 'hard-and-fast rules of evidence by which to judge causation', and emphasized that that the nine 'view points' were neither necessary nor sufficient to establish causation. Although Rothman & Greenland, (2005); Kundi (2006) and Swaen & van Amelsvoort, (2009) agree that the Hill Criteria still remain an essential components for causal inference, there is still a debate on the weight that should be given to each of the nine criteria and as a result, attempting to prove causality firmly or conclusively using this set of criteria is difficult (Swaen & van Amelsvoort, 2009).

3.4.3 GAPS IN THE LITERATURE

While the majority of epidemiological studies on nitrates in drinking water and thyroid disorders are ecological and have been conducted with nitrate data from public supply, well designed epidemiological studies are required with nitrate data from PWS where the risk of thyroid disorder (goitre) may be higher give that the level of nitrates in PWS is usually higher than in public water. Also, more studies are required on hypothyroidism; hyperthyroidism and thyroid cancer as the number of studies on these outcomes are currently too few to enable a reasonable

conclusion to be drawn. In the majority of the studies, there was no individual exposure assessment as nitrate levels in drinking water were obtained from records of Regional Inspectorate for water or Public Health Protection Board and used as a proxy for individual exposure. There was no measurement of nitrate levels in tap - water at the places of residence or the amount of tap - water intake by the cases and/-or control. More studies with individual exposure assessment are required to determine the level of nitrates in drinking water at the place of residence of the cases and control groups and the amount of individual tap-water intake per day. Although causality has not been firmly established between exposure to nitrates in drinking water and goitre, the risk assessment framework can be used to estimate lifetime excess risk of goitre in East Anglia given widespread nitrate contamination of drinking water, particularly in PWS in the region.

3.5 CONCLUSION

Review of animal and epidemiological studies suggests that exposure to nitrates in drinking water is associated with moderate - to - mild iodine deficiency; hypothyroidism; hyperthyroidism and goitre. Following a meta- analysis, the weight of evidence is strongest for thyroid hypertrophy or goitre (effect estimate, OR = 3.13); weak for subclinical hypothyroidism (OR = 1.23) and weakest for clinical hypothyroidism and hyperthyroidism (clinical and subclinical). The effect estimates shows that the risk of goitre is more than 3times higher in the exposed group than control group and suggest that exposure to nitrates in drinking water is a risk factor for goitre. While the goitrogenic effect of nitrates is more on children and women, especially pregnant women, the effect of subclinical hypothyroidism is also more on children (particularly infants) than adults. Although the majority of the anti - thyroid effects of nitrates was reported at nitrates levels equals to or greater than the drinking water standard of 50mg/l, some of the effects

also occurred at nitrate concentrations below 50mg/l. The effect of nitrates on the thyroid gland is strong if there is dietary iodine deficiency and weak if dietary iodine intake is optimal. Infants are more sensitive to iodine inhibitors (including nitrate) than adults and therefore require more iodine intake in order to produce more thyroid hormones during shortages. This is because of the short half-life of thyroid hormones in infants (approximately 3 days) and their inability to store thyroid hormones for more than a day for use during shortages like in adults. Although the number of studies on hypothyroidism (clinical or subclinical) is too few to enable a firm conclusion to be drawn, the evidence for subclinical hypothyroidism is supported by result of animal studies.

The mechanism of nitrates on the thyroid gland stems from the ability of nitrate ions to inhibit iodine uptake by the thyroid gland by binding to the sodium – iodide symporter (NIS) on the surface of the thyroid follicles resulting in a decreased in the amount of iodine available in the thyroid gland and consequently resulting in decreased thyroid hormones (triiodothyronine (T3), thyroxine (T4)) production; hypothyroidism and increased level of thyroid stimulating hormones (TSH). Chronic stimulation of the thyroid gland to produce more hormones by the TSH during shortages can result in thyroid hypertrophy (goitre); hyperplasia and carcinoma. Although the number of studies are too few to enable a meta- analysis on thyroid carcinoma, the relationship between nitrate exposure and thyroid cancer is biologically plausible and may be attributed to the ability of nitrate metabolite (NO_x) to react with amines or amides (nitrosation) in food e.g. red meat to form NOCs. The majority of NOCs are associated with cancer at various organ sites including the thyroid in animals and humans. Although exposure to nitrates in drinking water in the presence of nitrosation precursors can promote the production of NOCs and cancer, intake of antioxidants e.g. vitamin C can inhibit the production of NOCs and the induction of cancer. Also adequate intake of iodine can counteract the effect of nitrates on the thyroid.

CHAPTER FOUR

4.0 RESEARCH METHODOLOGY

4.1 AIMS AND OBJECTIVES

The aim of this study is to evaluate the relationship between exposure to nitrates in drinking water and thyroid disorders with reference to Private Water Supplies (PWS) and public water supplies and to quantify any risk in East Anglia, UK.

The specific objectives are:

1. Hazard Identification.

- To review available case-reports, epidemiological and experimental animal studies for any evidence of thyroid disorders (including hypothyroidism; hyperthyroidism; thyroid hypertrophy; goitre; hyperplasia; carcinoma etc.) following exposure to nitrates in drinking water.
- To characterise the route(s) of exposure to nitrates.
- To characterise the mechanism of action of nitrates on the thyroid gland.
- To characterise the strength of any association between nitrate exposure in drinking water and any thyroid disorders.
- To determine whether any association is causal.

2. **Dose - Response Assessment.**

- To describe the relationship between exposure to nitrate in drinking water and thyroid hypertrophy (goitre) from epidemiological studies.
- To describe the shape of the dose-response curve (linear or non-linear).
- To determine the effect of nitrate exposure on the thyroid gland at low- dose.
- To synthesise the evidence.

3 Exposure Assessment

- To estimate the concentration of nitrates in public water and PWS in East Anglia by collecting and analysing data on nitrate concentrations from water companies and Local Authorities respectively.
- To determine the magnitude, frequency and duration of exposure to nitrates in drinking water.
- To determine the amount of tap-water intake by the population in the area administered by Suffolk Coastal District Council.
- To determine whether there is any difference in the amount of tap-water intake between public water and PWS users.

4 Risk Characterisation

- To estimate the population attributable faction (PAF) or lifetime excess risk of goitre in East Anglia following exposure to nitrates in drinking water by integrating information from dose - response analysis and exposure assessment.
- To characterise the strength and limitations of the study.
- To characterise uncertainties associated with the risk estimates.

- To evaluate the appropriateness of the current nitrate drinking water standard of 50mg/l (set to protect against infantile methaemoglobinemia) in protecting against thyroid hypertrophy (goitre).

4.2 QUANTITATIVE RISK ASSESSMENT

Risk assessment is defined as the process of using all available scientific information to determine the ability of a chemical or substance to cause adverse health effect following exposure to an individual or a population (National Research Council (NRC) 1983; 2008). In other words, it is essentially the collection and evaluation of all relevant information about the potential health effect of a substance or agent in a specific situation in a logical and objective manner. The main objective of human health risk assessment is to determine evidence –based action that may be necessary for public health protection from substances, agents or developmental projects that may have the potential to affect health (Brownson et al 1999; Environmental Heath Standing Committee (enHealth), 2004). It is a means of evaluating public health evidence in order to determine when public health action is required (Brownson et al, 1999). It involves the evaluation of the nature and magnitude of adverse health outcomes from past exposures and prediction or estimation of the outcome from future exposures (United States Environmental Protection Agency (USEPA, 1990a). In the regulatory context, the process can be used to approve new chemicals or substances prior to marketing or release into the environment and the re-evaluation of existing substances (Clegg et al, 1996). The output of the risk assessment exercise provides evidence that can be used to make a decision on how best to manage the risk (Spickett et al, 2006). The four stages of risk assessment are hazard identification; dose - response evaluation; exposure assessment and risk characterisation.

- **HAZARD IDENTIFICATION**

This objective has been completed (Chapter Three, pgs. 50-102). It involved a review of animal and epidemiological studies for any evidence of thyroid disorders. It also involved characterising the route(s) of exposure; the biological properties (including toxicokinetics and toxicodynamics) of nitrates as well as the nature and strength of any evidence of association and determining whether any association is causal. A review of animal and epidemiological studies suggests that exposure to nitrates in drinking water is associated with moderate - to - mild iodine deficiency; hypothyroidism; hyperthyroidism; goitre and thyroid cancer. However, following a meta - analysis, the weight of evidence is strongest for goitre (effect estimate, OR = 3.13); weak for subclinical hypothyroidism (OR = 1.23) and weakest for clinical hypothyroidism and hyperthyroidism (clinical and subclinical). Although causality was not firmly established, the risk assessment framework can be used to determine any excess risk of goitre in East Anglia following widespread nitrate contamination of drinking water sources. Hazard identification is the first stage in the risk assessment process and is used to determine (on the basis of available data, including animal and epidemiological studies as well as case-reports) whether a substance or an agent has the ability to cause or result in increase in adverse health effect following exposure to an individual or a population (Abernathy and Roberts, 1994).

- **DOSE – RESPONSE ASSESSMENT**

The relationship (linear or non- linear) between exposure to nitrates in drinking water at various doses and any effect on the thyroid gland will be determined. Also the effects of exposure to nitrates on the thyroid gland at low doses (i.e. doses below the lowest observed adverse effect level reported in epidemiological studies) will be examined. Dose-response evaluation examines

the relationship between exposure of an agent or substance and the incidence of adverse health effects in an exposed population. In other words, it is the quantitative assessment of the relationship between exposure of an agent and a health outcome from a given set of data and a prediction from the data of future effects (Roberts and Abernathy 1996).

- **EXPOSURE ASSESSMENT**

Exposure assessment is the process of determining the magnitude, frequency and duration of exposure of a substance or an agent to an individual or population (Gerba, 1996; Ball, 2006). Also, it involves the determination of the route of exposure, the size, composition and characteristics of the exposed population as well as the determination of the most sensitive subgroup (Roberts & Abernathy, 1996; Sand, 2005). Exposure can be measured directly or indirectly. Direct measurement involves either measuring exposure concentrations of a substance to a target individual at the point and time of contact; biological monitoring or the use of a biomarker (Ott et al, 2007). Whilst point of contact approaches indicate the total concentration reaching the target individual; biological monitoring or the use of biomarkers indicate the concentration of the substance within the body after the exposure has taken place (Ott et al, 2007). Although the direct approach gives a more accurate exposure data, it is invasive, expensive to conduct and may not always be feasible especially in a population exposure study (Ott et al, 2007). An indirect approach, on the other hand, can be conducted quantitatively and/or qualitatively (Sand, 2005; Ball, 2006) and involves measuring the concentration of the substance or chemical in an environmental medium (e.g. air, water, food or soil) in order to predict the exposure distribution within the population (Ott et al, 2007). Whilst this approach is more cost effective and less invasive to the population than the direct approach, the results are sometimes subject to error as it does not consider whether actual exposure took place. Also there

may be some error in the measured concentration of the substance or chemical in the exposure medium (Ott et al, 2007).

This assessment followed the indirect approach. It was conducted quantitatively and involved collection of data on nitrate concentrations in drinking water (public and private water supplies) from water companies and local authorities in East Anglia. Information on the amount of tap-water intake by the population in the region was obtained via a questionnaire administered on some randomly selected households in the region.

- **RISK CHARACTERISATION**

Risk characterisation is the final step in the risk assessment process. It combines information from dose-response and exposure assessment in order to quantitatively estimate the health risks of a substance or agent in a population. It involves a description of the nature, extent, and severity of risks and making a conclusion as to whether exposure to a substance or agent is associated with any increase in the incidence of a health effect or a level of exposure could be identified that is considered tolerable (USEPA, 1986; Roberts & Abernathy, 1996). Here, any assumptions or uncertainties associated with the risk estimates are characterised.

4.3 DISCUSSION

Risk assessment provides an understanding of the sources and pathways of contaminants in the environment which is an important step in addressing (and reducing) uncertainty associated with estimating the likelihood of exposure to such contaminants in the environment (Ritter et al, 2002). A good understanding of the sources and pathways of contaminants “strengthens our

ability to quantify effects through accurate measurement and testing or to predict the likelihood of effects based on empirical models" (Ritter et al, 2002: pg.1). Therefore, understanding the source(s), concentration and exposure pathway of nitrates in drinking water and estimating the risk of thyroid disorders in East Anglia not only forms the basis of risk characterisation, but also provides information that may be used by policy makers like DEFRA and DWI in articulating regulatory and other risk management options aimed at improving water quality and protection of public health. The information may also be useful to the Environment Agency in the regulation and processing of licences for drinking water abstraction especially for private use.

Risk assessment is a useful tool in public health decision making and is widely used internationally in expressing risk and making regulatory decisions on a number of chemicals and environmental contaminants such as pesticides, heavy metals, poly-aromatic hydrocarbons (NRC, 1983 & 2008; Ritter et al, 2002). It has been used by WHO, USEPA, the European Environment Agency (EEA) and numerous regulatory agencies and public health institutions around the world in the management of air and drinking water quality as well as in contaminated land management (NRC, 2008). The process has also been used in many OECD (Organisation for Economic Co-operation and Development) countries to set environmental priorities, to guide legislation, choose regulatory and risk management options and perform cost-benefit analysis (World Bank, 1998). In the UK, risk assessment has been used to set emission targets (from motor vehicles and or industries) for air quality management; Soil Guideline Values (SGVs) for contaminated land management and regulation of pesticides (DEFRA, 2009).

Although risk assessment has over the years been used as a tool in evidence - based public health decision making especially in expressing risks (Ritter et al, 2002); setting environmental standards or guideline values; regulation of hazardous chemicals, substances and or practices and articulating risk management options (Briggs, 2008; NRC, 2008), concerns have been expressed

about its continued use in public health decision making (Silbergeld, 1993; Montague, 2004; Michaels, 2008). These concerns stem from the length of time it takes to complete the risk assessment of some chemicals or practices (up to 10 years or more in some cases); lack of relevant data; uncertainties associated with available data and the science underlying the process (NRC, 2008). Also there is the issue of lack of stakeholder's involvement in the risk assessment of substances or practices designed to address public concerns, which could lead to lack of confidence in risk management options. To address these concerns, USEPA in collaboration with the NRC set up a committee in 2007 to come up with ways to improve risk assessment in order to make the process more useful and acceptable to policy makers, risk managers and stakeholders (NRC, 2008). Although the committee recommended improvements in the approaches used in risk assessment and the science underlying it, as well as its utility, they however advocated for the retention of the risk assessment concept in policy decision making especially in public health protection. They also recommended that the process should not be seen as end itself but as means of evaluating various risk management options (NRC, 2008; Levy, 2009).

4.3.1 EVIDENCE - BASED PUBLIC HEALTH (EBPH)

The goal of public health is to create conducive environment or conditions in which people can be healthy 'in order to advance the society's collective interest in promoting and preserving good health' (Institute of Medicine (IOM), 1988). In other words, public health is about "doing whatever it takes to prevent unnecessary disease, disability or premature death" (Petersen & Alexander 2002.pg 2). There is no doubt that advances in public health in the last three-four decades have contributed to gains in life expectancy and improvements in the air we breathe, the water we drink and the food we eat (Petersen & Alexander, 2002). Also, they have contributed to

improvements in sewage treatment and disposal; reductions in tobacco use; injury prevention; control of infectious diseases through immunisation and other interventions aimed at public health protection (United States Centre for Disease Control (CDC), 1993a; Petersen & Alexander, 2002). However, in spite of these achievements, humanity continues to experience many public health challenges and in order to continue to adequately address these challenges, evidence- based strategies are required in public health policy decision making.

The principle of using evidence - based public health (EBPH) in decision making has emerged in recent times as a standard public health practice (Brownson et al, 1999; 2009). EBPH is defined as, "the use of scientific evidence and data in the development, implementation, and evaluation of effective programs and policies in public health" (Brownson et al, 1999 pg. 87). According to Hess (2014. pg1117), "it is a process whereby evidence related to the nature and magnitude of public health problems and interventions to address them is systematically assembled, evaluated, and integrated into public health decision making". The first step in the process of EBPH is 'problem assessment' or 'needs assessment' (Hess et al, 2014). Needs assessment aims to identify the public health concerns or 'needs' of a population and determine how best to meet those needs, using available information and data. A "need" is a discrepancy or gap between "what is" and "what should be" (Office of Migrant Education (OME), 2001.pg2). According to Wright & Cave (2013), needs assessment is a systematic method of identifying the public health and/or social care needs of a population and making recommendations for changes to meet those needs. It is the process of using data and the opinions of stakeholders to establish a consensus on public health concerns or priorities, as well as the most cost-effective way to address those needs (Petersen & Alexander, 2002). A 'Need' could be perceived, expressed or relative (Bradshaw, 1972; Kettner et al, 2008). Whilst perceived 'Need' relate to what people think about their needs (which may vary between individuals); expressed 'Need' relate to the number of people who

have sought help or information about their needs (thus translating feelings into action); relative Need is concerned with equity, taking into consideration variations in population (Bradshaw, 1972; Kettner et al, 2008).

According to Brownson et al (1999), tools used in EBPH to determine public health actions include risk assessment; economic evaluation; public health surveillance and expert panel consensus conference reports. This may involve a meta-analysis (quantitative integration) of individual study results and cost- benefit analysis of any risk management options. EBPH has been used to develop strategies for climate change adaptation (Hess et al, 2014); injuries in the U.S military (Jones et al, 2010); physical inactivity in Europe (Cavill et al, 2006); and low level of health literacy among mothers (Levandowski et al, 2006). Although there is still a debate about EBPH in concept and applicability as critics have suggested that it can be used as a justification for taking no action and/-or to cut public health budgets (Coburn & Poland, 1996; Poland et al, 1998; Nadav & Dani, 2006), it is widely recognised and still used in public health decision making (Brownson et al, 2009; 2013).

4.3.2 PUBLIC HEALTH STRATEGY IN ENGLAND

EBPH is at the centre of the new public health strategy in England. The White Paper, Healthy Lives, Healthy People (Department of Health (DoH), 2010a), published by the government in 2010 set out a framework for a Public Health Strategy for England which focused on three major areas:- health protection; health improvement; and the quality of health services provision. It followed the earlier White Paper, published in July 2010 (DoH, 2010b), which focused predominantly on the wider issues of health and social care. The document which emphasised individual responsibility for heath also acknowledged the implications of wider social, economic

and environmental factors on health and well-being. The Public Health White Paper embraced a number of principles and focuses emphasis on professional leadership and the use of evidence in determining health issues and the best way to address those issues (evidence - based public health).

EBPH is also at the centre of the Health and Social Care Act (2012) which amended the Local Government and Public Involvement in Health Act (2007). The 'Act' which required the production of local Joint Strategic Needs Assessments (JSNAs) and Joint Health and Wellbeing Strategies (JHWSs) aimed to "develop local evidence - based priorities for commissioning which will improve the public's health and reduce inequalities. Their output, in the form of evidence and analysis of 'Needs' and agreed priorities will be used to help determine which actions Local Authorities, the local National Health Service (NHS) and other partners will take to meet health and social care needs and to address wider determinants that impact on health and well-being" (DoH 2012.pg4). The Government in setting out this strategy believed that the previous approach and system in public health was not seizing available opportunities for better health in order to reduce inequalities in health. Therefore a new approach was required that would empower local communities, enable professional freedom and unleash new ideas based on the evidence of what works, while ensuring that the country remains resilient to and mitigates against current and future health threats. Localism is at the centre of the Public Health Strategy which transferred Public Health Departments from Primary Care trusts (PCTs) to Local Governments in 2013. Given the susceptibility of PWS to chemical and microbial contamination, the DWI recommended that the safety of PWS should be incorporated into each Local Authority's Public Health Strategy DWI (2014) in order to protect the population from health threats posed by contaminants in drinking water.

4.4 DESCRIPTION OF THE STUDY AREA

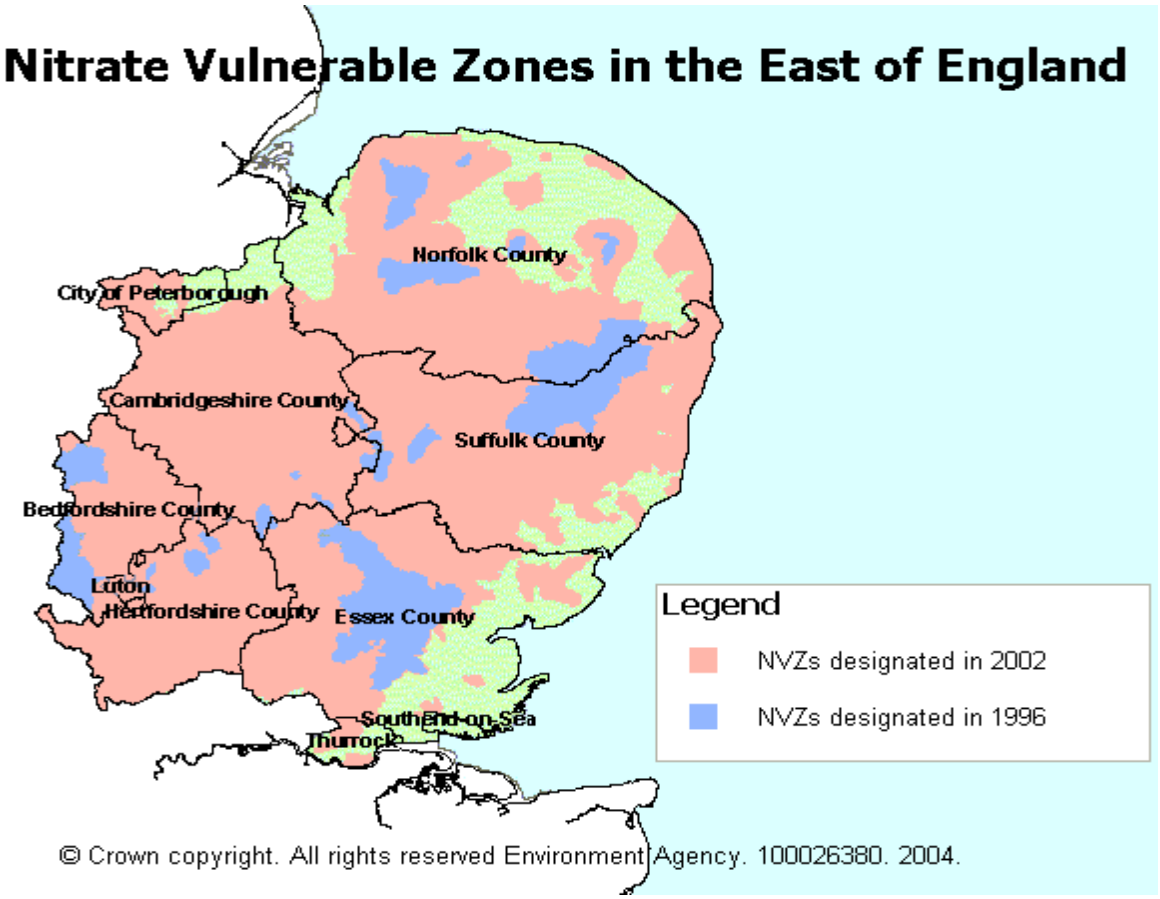
East Anglia (Figure 15) is regarded as the second largest region in England. It borders London in the south, the Midlands in the west and remote coastal and rural areas in the north and east. The region covers an area of 19,120 km^2 with a population of about 5.8 million in 2011 (Office of National Statistics (ONS) 2013). Six counties (Bedfordshire; Cambridgeshire; Essex; Hertfordshire; Norfolk and Suffolk) and four unitary authorities (Luton; Peterborough; Southend-on- Sea and Thurrock) make up the region.

Figure 15: Map of England depicting East Anglia (Source: EEDA 2009)

There are 44 District and Borough Councils in the region. Land use other than residential is predominantly agricultural (over 75 per cent of the land is used for agriculture). East Anglia is one of the Nitrate Vulnerable Zones (NVZs) of the UK. The region was designated a NVZ (figure 16) by the UK Government in compliance with European Union (EU) Nitrate Directive

91/676/EEC, aimed at reducing nitrate pollution of controlled waters (including drinking water sources) from agricultural land (DEFRA 2005).

Figure 16



About 77 percent of agricultural land in England was designated NVZs in 1996 & 2002 (DEFRA 2004; 2009) the majority of which, is in East Anglia (DEFRA 2005, Davies 2000). The region is also considered to be one of the driest parts of the UK, with winter rainfall as little as 150mm (Davies 2000). Thus, nitrate leaching of as little as 15kg/ha could result in nitrate concentrations in drinking water sources in breach of the EU limit due to little or no dilution (Davies 2000). Four water companies (Anglian Water; Essex & Suffolk; Cambridge; and Affinity formerly Tendring Hundred) supply the majority of the population with drinking water while the

remaining population, located in mostly rural parts of the region obtain their drinking water from private water sources e.g. wells and boreholes (DWI 2004).

4.5 SOURCES OF DATA: DATA COLLECTION

4.5.1 WATER COMPANIES (PUBLIC WATER SUPPLY)

Data on nitrate concentrations in public water supply (for exposure assessment) was collected from all four water companies serving the region. This was done via a letter (Appendix 3) sent to the Water Quality Manager of each of the water companies in the region in October 2010 requesting information on nitrate levels in drinking water recorded in their various areas of operation in the previous 10 years. Also requested was information on drinking water source (groundwater or surface water); number of samples taken each year and the number of people served in each water supply zone. Water supply zones are operational areas of a given population within a water company's operational boundary and each zone may be served with water from a particular treatment works or reservoir. However, in certain circumstances, a water zone may receive water from more than one reservoir or source in a year.

It must be noted that nitrate data on public supply water provided to DWI by water companies as required by the legislation are only compliance data (50mg/l NO_3) and does not give the actual nitrate level in water sources before blending. The process of blending as practised by water companies involves mixing water with elevated nitrate levels and water with low nitrate levels in

order to achieve the drinking water standard of 50mg/l. However, for the purposes of this study, actual nitrate concentration in water sources (before blending) was collected from water companies, as this gives a clearer picture of the concentration of nitrates in drinking water in East Anglia.

4.5.2 LOCAL AUTHORITIES

Data on nitrates levels in PWS was collected from Local Authorities in East Anglia following a letter and a questionnaire (Appendix 4) sent to the Environmental Health Manager of each of the 44 Local Authorities in East Anglia. The questionnaire asked for information on the number of PWS in their area; nitrate levels recorded in each supply and the number of households served. The number of households served by each PWS enables the number of people in the household to be estimated. According to the UK 2011 census statistics, the average number of people per household in the UK is 2.3; in England (including East Anglia) the average is 2.5 people per household (ONS, 2013).

Table 3 (Appendix 5) shows the average nitrate concentration recorded in public water supply and PWS in East Anglia as provided by Water Companies and Local Authorities and the population served. Whilst Anglian Water (the dominant water company in the region) provided data from 2001 to 2010 (part), the other three companies provided data from 2000 to 2009. The data shows that the average nitrate concentration recorded by water companies in the region in the 10 years ranged from <0.1-to- 56.4mg/l and the total population served in the same period were 2,836,408.

Of the 44 Local Authorities contacted for nitrate concentration in PWS, 36 (82 per cent) completed and returned their questionnaire. Of the 36 Local Authorities that completed and returned their questionnaires, six indicated that they had no private water supplies in their areas, leaving the number of Local Authorities with relevant data to 30(68 per cent). Whilst data on the concentration of nitrates in PWS in the region ranged from 0.3 -to- 466.6mg/l, the number of people served was 13,510. Thus, the total number of people in the exposure assessment was 2,849,918 (public water users 2,836,408; PWS users 13,510).

4.5.3 DATA ON TAP - WATER INTAKE

Data on the amount of tap-water intake per day by individuals in the study area was obtained via a questionnaire (Appendix 6) sent to 100 residential addresses in Suffolk Coastal District Council. 50 copies of the questionnaire were sent to residential addresses with known public water supply and 50 copies were sent to residential addresses with known PWS. All the residential addresses were selected at random.

Suffolk Coastal District Council is one of the Local Authorities in East Anglia. It covers an area of about 320 square miles and had a population of about 124,700 in 2011(ONS 2013). The district is predominantly rural and land use other than residential is predominantly for agricultural and horticulture. Two water companies, Anglian Water and Essex & Suffolk Water supply the majority of the district's drinking water and there are about 350 private water supply locations, serving about 652 houses in the district. These supplies are covered by The Private

Water Supply Regulation (2009). The district was selected for this survey because records of PWS including their locations, the concentration of nitrates in these supplies and the addresses served are well maintained and therefore it was more convenient to administer the questionnaire to the households. The use of a questionnaire in collecting information on tap-water intake has been validated by Kaur et al (2004) and Mons et al (2007) and has been judged to have a higher response rate than diaries (Kaur et al 2004).

4.6 ETHICAL APPROVAL

Ethical approval was sought and obtained in September 2010 (Appendix 7) from the ethics committee of the School of Health and Human Sciences, University of Essex before the data collection exercise. Also approved by the Ethics Committee was the questionnaire used to obtain information on tap-water intake from the population in Suffolk Coastal District Council (Appendix 6). Permission was also sought and obtained from the Principal Environmental Health Officer, Suffolk Coastal District Council for the use of their nitrate data from PWS and before administering the questionnaire on the residents. No ethical approval was required by the water companies as the Water Supply (Water Quality) Regulations 2000 (as amended) have provisions for a public register. To maintain confidentiality, no individual names were collected as part of the data collection exercise. Although addresses and postcodes were required for the questionnaires to be sent and for follow – ups, these were kept securely locked in a filing cabinet and were not published as part of this study.

4.7 SUMMARY

Risk assessment is a valuable tool in evidence - based public health decision making and has been used over the years to determine actions aimed at public health protection, including regulation of hazardous chemicals, substances or practices that may affect human health. The four stages of the process (hazard identification; dose response assessment, exposure assessment and risk characterisation) address different questions that can be used to estimate excess risk of goitre in East Anglia as a result of exposure to nitrates in drinking water. Although risk assessment has been criticised as sometimes not being able to meet the needs of risk managers or policy makers, it still remain a very important tool in EBPH in formulating public health policy.

CHAPTER FIVE

5.0 DOSE-RESPONSE ASSESSMENT

5.1 INTRODUCTION

The purpose of dose – response assessment is to determine the relationship between exposure to a substance or chemical and a health outcome (Ball, 2006). In other words, it is a determination of the level of effect a substance would have on a particular organ at different doses of exposure. Given that the weight of evidence assessment suggests that exposure to nitrates in drinking water is strongest for thyroid hypertrophy (goitre), this outcome was selected for dose- response assessment in order to quantitatively estimate the excess risk in East Anglia. Dose-response relationship on thyroid hypertrophy (goitre) was conducted with epidemiological data only.

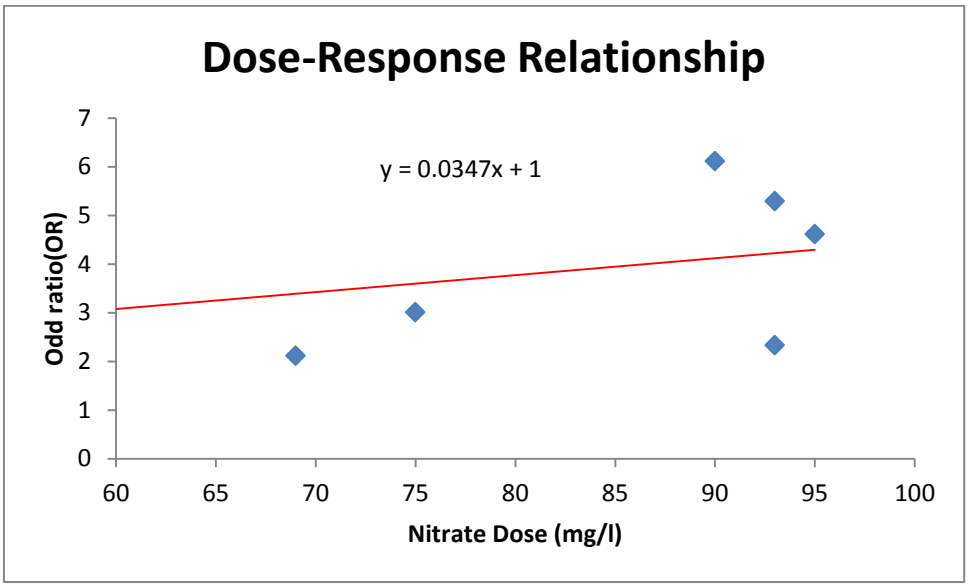
Although epidemiological and experimental animal studies can be used in hazard identification and dose - response assessment (World Bank Group, 1998; IRR 2003), epidemiological data, when available, is more relevant than animal data and therefore preferred in dose-response assessment (Smith, 1988; IARC, 1994; Hertz – Picciotto, 1995; Shore, 1995; Samet et al, 1998; World Bank Group, 1998; IRR, 2003). This is because, the level of uncertainty or magnitude of error in risk estimates derived from epidemiological data is far lower than that derived from animal data (Smith, 1988). According to Hertz - Picciotto (1995) and Samet et al (1998), uncertainties associated with extrapolation from animals to humans are larger when compared

with intra - human extrapolation. Also, whilst exposure experience by animals is a poor representation of human exposure, the context of exposure also differs between animals and humans. Another reason is that genetic diversity and variability in humans is better represented in human studies than in animal studies (Hertz – Picciotto, 1995). However, the use of epidemiological data in dose - response assessment has been criticised by various authors (Occupational Safety and Health Administration (OSHA), 1980; Doll, 1985; USEPA, 1986; Wartenberg & Simon, 1995). This is because epidemiological studies are judged to be of lower sensitivity and of low statistical power (Doll, 1985; Wartenberg & Simon, 1995); limited by exposure misclassification bias or confounding factors; and failure to provide a controlled, randomised, experimental situation compared to animal data (OSHA, 1980; USEPA, 1986). Although the USA National Research Council in its commentary on air pollutants (NRC, 1994) gave preference to toxicological data in dose-response assessment because of the cost of conducting epidemiological research and the conflicting nature of epidemiological study results, epidemiological data has been used in a number of cases to identify hazards and establish dose – response relationship as in the case of radon gas exposure and lung cancer; asbestos and mesothelioma of the lungs; vinyl chloride and angiosarcoma of the liver; smoking (active and passive) and lung cancer (Samet et al, 1998). According to Hertz - Picciotto (1995: pg. 486), "when studies of sufficient quality are available for humans and animals, human data are preferable as a basis for extrapolation". However, when adequate epidemiological data is not available, animal data can be used in dose-response assessment if such studies demonstrate adverse health effects (IARC, 1994; Hertz – Picciotto, 1995).

5.2 DOSE - RESPONSE ANALYSIS

Dose-response assessment involved fitting a regression line to epidemiological data in Gatseva et al, 1997 & 1998; Gatseva & Argirova 2005; 2008a & b; Tajtakova et al (2006); Radikova et al (2008) and the relationship between nitrate doses and the odds ratio (response) described. Figure 17 shows the relationship between nitrate doses in drinking water and thyroid hypertrophy (goitre) in exposed individuals in the epidemiological studies. The assessment was conducted using the LINEST function in Microsoft Excel. Whilst the x-axis represents the doses of nitrate in drinking water in the study areas, the y-axis represents the response, i.e. relative risk expressed as odds ratio (OR). The intercept was set at 1 to suggest that for zero dose, the risk is 1. The regression obtained was y = 0.0347x +1. The value of R^2 is 0.833. A comparison of the figures with SPSS analysis gave identical values but also some additional information. This showed that the independent variable (dose) statistically significantly predicts the dependent variable, F (1, 5) = 24.970, p < .004.

Figure 17: Dose- Response Relationship between Nitrates and Thyroid Hypertrophy (Goitre).



Whilst figure 17 represents the effect of nitrates in drinking water on the thyroid gland in some individuals within the range of doses reported in epidemiological studies in the study areas, it does not show the effect of nitrates at doses below the lowest observed adverse effect level (LOAEL) i.e. below 69mg/l and this can be extrapolated in order to estimate the risk of the goitre in the entire dose range. Low – dose extrapolation is important as it allows for risk estimation in the entire dose range of exposure of a substance in a population (Shepard et al, 1987).

5.3 LOW- DOSE EXTRAPOLATION

In order to extrapolate the effects of nitrates in drinking water on the thyroid from the high doses reported in the epidemiological studies to low - doses, a distinction must be made between substances exhibiting threshold or non - threshold effects. For some substances e.g. non-carcinogenic, where it is assumed that a threshold dose exists, below which, there is no appreciable toxic effect i.e. there is a range of exposure doses that can be tolerated by an organism without the expression of observable toxic effect, the no-observed adverse effect level (NOAEL) and reference dose (RfD) approach can be used to extrapolate risks from the high exposure doses to low exposure doses (Barnes & Dourson, 1988; Gerba 1996). The threshold dose is represented by the RfD and doses below or equal to the RfD are considered not to have appreciable health risks, while doses above the RfD are assumed to have some health risks (USEPA, 1990a; WHO, 1994). The RfD is the dose of a substance per unit body weight per day (mg/kg-bw/day) that is unlikely to pose appreciable risk to humans including the most sensitive group e.g. children and the elderly (Barnes & Dourson, 1988; Gerba 1996). It is estimated by applying uncertainty factors to the no-observed adverse effect level (NOAEL) or the lowest observed adverse effect level (LOAEL) where the former cannot be easily defined (Barnes &

Dourson, 1988). Whilst the NOAEL is the highest dose at which there is no adverse effect, the LOAEL is the lowest dose at which health effects begin to manifest (Barnes & Dourson, 1988). However, for some substances e.g. carcinogens, it is assumed that no threshold exists (i.e. it is assumed that there is the likelihood of some risk at every exposure dose), a mathematical model can be used in low - dose extrapolation of non-threshold substances (USEPA, 1990a; WHO, 1994).

Given that nitrate is known to be produced endogenously for some biological activities (Jaffe, 1981; Walker 1995; Hsia, 1998) such as antimicrobial activity (Hibbs et al, 1987; Fang, 1997; Addiscott & Benjamin, 2004); vasodilation (Bjorne, 2005); neurotransmission (Garthwaite, 1991); immune regulation (Hibbs, 1991), any threshold would already has been exceeded for these effects to occur. Therefore the effect of exogenous nitrate exposure via drinking water which have been reported to increase the concentration of endogenously produced nitrate as well as its toxicity (Berger et al 1997, Gupta et al 1998) suggest a non- threshold effect. As noted by Welshons et al (2003), "exogenous chemicals (e.g. endocrine disruption chemicals (EDCs) modulate a system that is physiologically active and thus is already above threshold, contradicting the traditional toxicological assumption of threshold for endocrine responses to toxic chemicals such as EDCs". The ability of nitrates in drinking water to inhibit iodine uptake by the thyroid gland by binding to the sodium - iodide symporter on the surface of the thyroid gland resulting in low thyroid hormone production; hypothyroidism or goitre (Alexander & Wolff, 1966; van Maanen et al, 1994; Tonacchera et al, 2004) suggests that nitrates is an endocrine disruptor. Given that the effect of nitrates is mediated by its metabolite, NO_x and not nitrite as previously thought (Addiscott & Benjamin, 2004), and the fact that NO_x is capable of exerting endocrine activity (Bryan et al, 2007; Elrod et al, 2008; Ghasemi & Zahedias, 2011)

further supports the endocrine disrupting potentials of nitrates. There is no threshold for EDCs (Sheehan & vom Saal, 1997; Crews et al, 2000).

Mathematical models are usually used in low - dose extrapolation of non-threshold substances because of difficulties in describing the shape of the dose-response curve (linear, sublinear or supralinear) and estimating risks at low - doses (USEPA, 1989 and 2005; IRR, 2003; Ball, 2006). Although extrapolation from high exposure doses of chemicals usually used in animal studies or observed in epidemiological studies to low doses in order to establish a 'safe dose' for humans has been criticised (Brown & Salmon 1996) especially for EDCs (Sheehan et al, 1999; Welshons et al, 2003; vom Saal et al, 1997; Vandenberg et al, 2012), no alternative method has been proposed. Whilst it is neither the aim nor the objective of this study to establish a 'safe dose' for nitrates in drinking water, low - dose extrapolation using a mathematical model can be used to quantitatively estimate the excess risk of goitre in East Anglia, following exposure to nitrates in drinking water. Although no particular model has been identified as the most suited for low – dose extrapolation of non- threshold substances (Paustenbach, 1989; Sand 2005) since each model can provide varying results in the low dose range (Krewski & van Ryzin 1981), it has been suggested that models that incorporate a linear relationship between the lowest observed dose and the zero-dose are preferred (Paustenbach, 1989; USEPA, 1989; Sand, 2005; USEPA, 2005).

Given that figure 17 suggest that the independent variable (dose) statistically significantly predicts the dependent variable, OR, ($F(1, 5) = 24.970$, $p < .004$, the equation of the regression line can be used in the low- dose extrapolation of the risk of goitre from the LOAEL.

$y = mx + 1$ **(Equation 1)**

Where:

y = relative risk (RR)

m = slope of the dose – response line (slope factor (q_1^*))

x = chronic daily intake (CDI) nitrate dose in drinking water

1 = a parameter that describes the background risk

The slope of the regression line (0.0347) will be applied to the daily dose (chronic daily intake) of nitrates in drinking water in the population in East Anglia in order to estimate the risk (unit risk or risk per specific dose) of goitre. The slope of the dose-response curve is called the potency factor (PF) or the slope factor (q_1^*) (Gerba, 1996; Roberts & Abernathy, 1996). The slope factor or potency factor is the risk produced by a lifetime average dose of 1mg of a chemical or substance per kilogram body weight per day (USEPA, 1990; Gerba, 1996). The slope factor (q_1^*) is usually incorporated in a selected model in order to estimate quantitatively the RR or probability of cancer risk per specific dose (risk specific dose (RSD)) of the exposure substance (USEPA 1986; 1989). This is done by multiplying the slope factor (q_1^*) with the chronic daily intake of the chemical (Roberts & Abernathy, 1996). Chronic daily intake (CDI) is defined as intake of a chemical over an average time of 70 years (lifetime), expressed in mg/kg-bw/day (Roberts & Abernathy, 1996; USEPA, 1989).

The slope factor derived from epidemiological studies (source populations) will be applied to the CDI in order to derive the RR of thyroid hypertrophy (goitre) in the target population (East Anglia). The slope factor derived from epidemiological studies (source population) where data is available can be applied to a different (target) population lacking their own data in order to estimate the health risks of a substance or an agent (Steenland &Armstrong, 2006; World Bank Group, 1998). This approach usually referred to as 'benefit transfer' (World Bank Group, 1998) has been used by Romieu et al (1990) to estimate the health effects of air pollutant (total suspended particulates (TSP) in Latin America where few epidemiological studies exist and the dose-response function has been calculated from epidemiological studies (found in the literature) conducted elsewhere on TSP. Although this practice can provide a rough estimate of risks, it can also introduce uncertainties in the risk estimates if there are significant differences in exposure prevalence, age composition of the exposed individuals, and individual variability between the 'source' population and the 'target' population (World Bank Group, 1998). However, given widespread nitrate contamination of drinking water sources in East Anglia; paucity of published epidemiological studies on the effects of nitrates on the thyroid gland in the UK (including East Anglia); similarities in exposure prevalence and age composition of exposed individuals between the 'source' population and the 'target' population in East Anglia, there is the rationale for this approach (benefit transfer) to be used to estimate the risk of goitre in East Anglia following exposure to nitrates in drinking water.

5.4 DISCUSSION

Dose-response relationship (figure 17) shows the effect of nitrates on the thyroid gland at different doses in a study population and suggests that the effect of nitrates on the thyroid at a

given dose or set of doses cannot be used to predict the effect of other doses hence the basis for extrapolation from the LOAEL (69mg/l) in order to estimate effects at low doses. The concept of low - dose effects has elicited considerable debate amongst researchers in recent years (Vandenberg et al, 2012, Fagin, 2012). The debate stems from the suggestion by some researchers (Vom Saal et al, 2007; Crain et al, 2007; Richter et al, 2007; Wetherill et al, 2007; Keri et al, 2007; Vandenberg et al, 2009; Vandenberg et al, 2012) that substances or chemicals with known endocrine disrupting capabilities or potentials can have effects on biological systems at low doses which can result in some disease conditions in humans and that the effects of these chemicals at high doses cannot be used to predict effects at low doses. Also, they suggested that non - monotonic dose-response relationship (NMDRs) (where response or effect increases and decreases as dose increases) are to be expected for some toxic chemicals (Kohn & Melnick, 2002; Conolly & Lutz, 2004) including endocrine disrupting chemicals (vom Saal et al, 1997; Welshons et al, 1999; Birnbaum 2012; Vanderberg et al 2012). Endocrine disrupting chemicals (EDCs) or endocrine disruptors is defined as a substance or mixture that alters the function(s) of the endocrine system and consequently cause adverse effects in an intact organism or its progeny or (sub) populations (IPCS/WHO, 2002). On the other hand low - dose effects, is defined in the USA National Toxicological Program (NTP, 2001) report, as "any biological changes occurring in the range of typical human exposures or occurring at doses lower than those typically used in standard testing protocols" (Melnick et al,2002.pg 427). Also, it has been defined as a dose or doses below the lowest dose at which a biological change (or damage) for a specific chemical has been measured in the past, *i.e.* any dose below the lowest observed effect level or lowest observed adverse effect level (LOAEL), or a dose administered to an animal that produces blood concentrations of that chemical in the range of what has been measured in the general human population (NTP 2001; Welshson et al, 2006). Adverse effect is defined by IPCS/WHO (2004) as impairment of functional capacity in an organism or impairment of capacity to compensate for

additional stress or increased susceptibility to the harmful effects of other environmental influences due to changes in morphology, physiology, growth, reproduction, development or lifespan of the organism. According to Vandenberg et al (2012), in a system that exhibits a non-monotonic dose response curve (especially an EDC), knowledge of the effect of one dose, or multiple doses, does not allow for assumptions to be made about the effects of other doses. The implications of this in regulatory toxicology is that the effect at high doses cannot be used to predict effect at low doses and any 'safe dose' determined from high doses does not guarantee safety at lower untested doses.

The low - dose effect hypothesis has been based on the evidence that natural endogenous hormones act at very low concentrations and that certain chemicals e.g. EDCs, which mimic natural hormones act at similar concentrations (Soto et al, 1994; Soto et al, 1995; Nagel et al, 1999; Welshons et al, 2003; Wozniak et al, 2005; Kochukov et al, 2009; Alyea & Watson, 2009; Vandenberg et al, 2012), suggesting that exposure to low - doses of these chemicals can contribute to some adverse health conditions in human population (Birnbaum, 2012). This could have implications for the current practice in risk assessment and regulatory toxicology where it is assumed that a threshold dose exist for these substances, below which, there is no observable or appreciable health risks. Although NMDR is regarded as a general biological phenomenon (Stebbing, 1982; Conolly & Lutz, 2004), opponents of the low - dose effect concept (Kyl et al, 2008; Ryan et al, 2010) have argued that whilst NMDR can exist for some chemicals, no adverse health effects have conclusively been linked to exposures to toxic chemicals at low - doses and that the current methods in regulatory toxicology and risk assessment are robust enough to protect against any health effects at low - doses (Kyl et al, 2008, Ryan et al, 2010). They also argued that, rather than adverse effects, low- dose of some toxic chemicals have demonstrated evidence of beneficial effects (hormesis) (Calabrese and Baldwin, 2001a) and can stimulate

some physiological processes such as growth and reproduction, whereas high doses have the opposite effect (Luckey 1975; 1980). NMDR has not been established for nitrates. However, exposure to low doses of endocrine disruptors have been linked to diseases such as obesity (Carwile & Michels, 2011; Hatch et al, 2010); diabetes (Grun, 2010); cancer (Soto & Sonnenschein, 2010); cardiovascular diseases (Akinbami 2010); reproductive and developmental disorders (Stillerman et al, 2008; Meeker & Stapleton, 2010); immune dysfunction (Miyashita et al, 2011) and neurobehavioral disorders (Swan et al, 2010).

In a workshop on low dose effects and non-monotonic dose - responses in relation to EDCs organised by the European Commission's Joint Research Centre (JRC) and the US National Institute of Environmental Health Science (NIEHS) in September 2012 in Berlin, Germany, participants agreed that NMDR exists for some chemicals and substances including EDCs over a certain dose range but there was no consensus on their toxicological consequences or implications in risk assessment. Although there was also a consensus that the definition of 'low dose' and 'adverse effect' need to be clarified, there was no consensus on whether EDCs should be treated as having a threshold or non - threshold effect. However, in a personal communication with some of the workshop presenters such as Peter Korytar (DG, EU Commission on Environment); Tryrone Hayes (University of California, Berkeley); Ana Soto and Linda Vandenberg (Tufts University), there was the view that the principles of endocrinology; mode of action of natural endogenous hormones and EDCs can be used to make a judgement as to whether EDCs exhibit a threshold or non- threshold effect (Personal Communication). According to Sheehan & vom Saal (1997) and Crews et al (2000), there is no threshold for EDCs. This observation may apply to nitrates based on their endocrine disrupting capabilities.

The assumption of linearity in the extrapolation of nitrates from the LOAEL to low-doses in order to estimate the excess risk of goitre in East Anglia is supported by the mechanism of nitrates on the thyroid gland. This stems from the ability of nitrate ions to inhibit iodine uptake by the thyroid gland by binding to the sodium – iodide symporter (NIS) on the surface of the thyroid follicles, resulting in iodine deficiency; low thyroid hormones production; increased thyroid volume; thyroid hypertrophy or goitre (Bloomfield et al, 1961 and 1962; Alexander & Wolff, 1966). According to the USA Environmental Protection Agency (USEPA), linearity at low- dose is to be assumed for substances whose toxicity is mediated by binding to a receptor (USEPA, 2000).

Given that the effect of nitrates on the thyroid gland when present in a mixture with other iodine inhibitors such as perchlorate and thiocyanate is additive, with no evidence of synergism or antagonism (Tonacchera et al, 2004), the assumption of linearity at low dose is consistent with the suggestion that the effect of substances e.g. genotoxic carcinogens that act in a dose-additive fashion is linear at low dose (California Department of Health Services 1986; USEPA, 1986; 2000). According to Crump et al (1976), any exposure affecting an already on-going process will lead to linearity at low dose. This hypothesis of 'additivity to background' was the basis for the justification of linear low - dose extrapolation of cancer risks for carcinogens and "if carcinogenesis by an external agent acts additively with any already on-going process, then under almost any model, the response will be linear at low dose" (Conolly & Lutz, 2004, pg. 155). This assumption is widely accepted (Krewski et al, 1995; Conolly & Lutz, 2004) given that DNA damage is sometimes unavoidable (Gupta & Lutz, 1999).

The assumption of linearity between the lowest observed effect levels (LOEL) or dose and the zero dose is further supported by receptor – ligand binding kinetics as explained in Welshons et al (2003). According to the authors, at low - doses, the relationship between a toxicant or hormone and its target receptor is linear and also results in a linear response or effect. But when the dose increases, the relationship between a receptor and a ligand as well as response or effect is nonlinear (Welshons et al, 2003). As noted by Conolly & Lutz (2004: pg.155), "the first interaction of a toxic agent and its primary biological target molecule follows the law of mass action, which results in a linear dose – response relationship; therefore, a linear default extrapolation at low - dose appears appropriate".

5.5 CONCLUSION

Animal and epidemiological data can be used in dose – response assessment but epidemiological data is preferred when available. This is because, the level of uncertainty or magnitude of error in risk estimates derived from epidemiological data is far less than that derived from animal data. Also, whilst exposure experience by animals is a poor representation of human exposure, the context of exposure also differs between animals and humans. Another reason is that genetic diversity and variability in humans is better represented in human studies than in animal studies.

The ability of nitrates in drinking water to disrupt iodine intake by the thyroid resulting in increased thyroid volume or thyroid hypertrophy (goitre) suggests that nitrates is an endocrine disruptor. The effect of nitrate exposure at various doses in the dose - response relationship suggests that the effect of nitrates on the thyroid gland at a given dose or set of doses cannot be used to predict effect at other doses. In other words, the effect of nitrates at high doses cannot be

used to assume or predict effect at low doses and this has to be extrapolated in order to estimate effects in the low - dose range. Although it is not known if nitrates exhibit a NMDR, the endocrine disruption capabilities suggest there may be no threshold dose for nitrates and any 'safe dose' set or predicted from effects at high dose may be misleading. There may be no threshold for substances whose mode of action is by binding to a receptor, such as nitrates.

The linear model can be used in low - dose extrapolation because nitrate doses (independent variable) statistically significantly predicts the dependent variable (i.e. relative risk, expressed as odds ratio). Also the assumption of linearity at low- dose is consistent with the mechanism of nitrates on the thyroid gland through disruption of iodine uptake. The assumption of linearity at low dose is further supported by the ability of nitrates to act in a dose additive fashion when present in a mixture with other endocrine disruptors. Low- dose extrapolation is important in risk estimation because it allows for comprehensive risk estimation across the entire dose range recorded or measured in a population. However, these are a lot of uncertainties in extrapolation outside the range of data.

CHAPTER SIX

6.0 EXPOSURE ASSESSMENT

An important step in assessing the risk of thyroid hypertrophy (goitre) in East Anglia as a result of exposure to nitrates in drinking water is to determine the concentration of nitrates in drinking water and the population exposed. It also it involves a determination of the amount of tap- water intake by individuals in the region. As stated in Section 4.5, information on nitrates concentration in public and private water supplies was collected from water companies and Local Authorities (respectively) in East Anglia whilst the amount of water intake was determined via a questionnaire sent to a subset of the population (Suffolk Coastal District Council).

6.1 DATA ANALYSIS

Table 3 (Appendix 5) shows the concentration of nitrates in drinking water in East Anglia which suggest that the concentration in public water ranged from <0.1 to 56.4 mg/l NO_3^- while the range in PWS was 0.3to 466 mg/l$NO3^-$. This covered a total population of 2,849,918. In order to determine the daily dose of nitrate intake (chronic daily intake (CDI)) over a lifetime of 70years, the general formula (Equation 2) for estimating chemical intake as formulated by the USEPA (1989b) was used.

$$\boldsymbol{CDI} = \frac{C \times CR \times EF \times ED}{BW \times AT} \qquad Equation\ 2$$

Where:

CDI = Chronic Daily Intake (mg/kg of body weight per day).

C = Average concentration of chemical (nitrate) in water during exposure period (mg/l).

CR = Contact rate or the amount of contaminated medium (water) contacted per unit of time (litres/day).

EF = Exposure frequency (days per year).

ED = Exposure duration (no of years of exposure).

BW = Body weight (kg).

AT = Averaging time (period over which the exposure is averaged – year/days).

This formulae, which is applicable to any length of exposure (e.g. sub-chronic or chronic) and with modifications to any route of exposure (Davis & Klein, 1996) has been used by the USA Environmental Protection Agency (USEPA) in the exposure assessment of contaminants from the Superfund sites (USEPA, 1989b). However, before using Equation 2, the contact rate (CR) has to be first determined. The contact rate is the amount of tap-water intake per day by individuals in the study population, usually expressed in (L/day).

6.1.1 CALCULATING THE AMOUNT OF WATER INTAKE (CONTACT RATE)

Information on the amount of tap-water intake in the study area was collected from residents in Suffolk Coastal District Council via a questionnaire (Appendix 4). The questionnaire asked participants to indicate the number of cups of tap-water, including for cold drinks (e.g. squash) and hot drinks (tea, coffee, hot chocolate) drunk each day at home. They were asked to express their daily intake of water as number of glasses (200 ml) or mugs (250 ml) per day. The number of glasses or mugs of water consumed each day in millilitres were later converted to litres (1000 ml is equivalent to 1L). The use of number of glasses or mugs as a measure to assess the volume of water consumed is considered the best way of estimating the amount of tap-water intake (DWI, 1996; Robertson et al, 2000; Gofti-Laroche et al, 2001; Dagendorf, 2003; Westrell et al, 2004; Mons et al, 2007) and is close to the everyday habit of the consumer (Mons et al, 2007). This method, however, does not take into account non-glass consumption of water such as ice-cubes, tooth brushing, taking medication or the use of glasses or mugs of different sizes. Non-glass consumption of water or use of glasses or mugs of different size can result in exposure misclassification bias (Mons et al, 2007).

Of the 100 addresses contacted for information on tap-water intake, 67 (67 per cent) completed and returned their questionnaires. Out of the 67 addresses that returned their completed questionnaire, 41(61per cent) were from households served by PWS while 26(39 per cent) were from households served by public water supply. This is however not surprising given the earlier interest shown on the research by people on PWS (personal discussion). The total number of people living in the 67 addresses that completed the questionnaire was estimated to be 168.

While 65(38.7 per cent) people lived in households served by public water supply, 103(61.3 per cent) lived in household served by PWS. Table 4 shows the characteristics of the respondents and the type of water consumed.

Table 4: Characteristics of Respondents

Age Group	**Households on Public Supply**		**Households on PWS**		
	No. of tap - water users	**No of bottled water users**	**No. of tap - water users**	**No. of Bottled water users**	**Total**
0-5	1	1	-	8	**10**
6-10	3	2	9	1	**15**
11-15	6	-	12	-	**18**
16-20	3	-	5	-	**8**
21-25	4	-	4	-	**8**
26-30	4	-	4	-	**8**
31-35	6	-	6	3	**15**
36-40	5	2	6	2	**15**
41-45	4	-	8	-	**12**
46-50	5	-	8	-	**13**
51-55	5	-	7	-	**12**
56-60	9	-	11	-	**20**
>65	5	-	9	-	**14**
Total	**60**	**5**	**89**	**14**	**168**
SEX					
Male	**23**	-	**48**	**6**	**77**
Female	**37**	**5**	**41**	**8**	**91**

Of the 65 people served by public supply, 60 reported drinking tap-water while 5 drank bottled water. In households served by PWS, of the 103 occupants, 89 indicated drinking tap- water while 14 drank bottled water. Of the 60 people who drank tap-water in households served by public supply, 23 were males while 37 were females. Among the 89 people that drank tap – water in households served by PWS were 48 males and 41 females. Of the 14 people who used bottled water in the households served by PWS, five were females aged 31-40 years while the rest (nine) were infants and children aged 0-10 years. Only two females aged 36-40 used bottled water in households served by public supply.

6.2 RESULT

Table 5 shows the amount of tap-water intake (contact rate) by the population in Suffolk Coastal area. Given that the amount of water intake per body weight of an individual changes over a lifetime (70years), a lifetime was divided into five periods: infant (≤1year); child (1-6years); child (7-12years); adolescent (13-18yrs); adults (19-70 years) (USEPA 1989b; Covello & Merkhofer, 1993).

Table 5: Amount of Water Intake (Contact Rate) by Age Group.

Age	Public supply (litre)	PWS (litre)	Average tap-water intake (litre)
Infants (≤1 year)	1.00	0.90	0.95
Child (1-6 years)	1.00	1.10	1.05
Child (7-12 years)	1.10	0.90	1.00
Adolescent (13-18 years)	0.875	0.75	0.8
Adult (19-70 years)	1.13	1.25	1.2
T- Test			
Mean	1.021	0.98	
Standard Deviation	0.1004	0.1956	
95%CI: (-0.1193, 0.2013) T- statistic = 0.417, p>0.05			

Analysis of tap-water intake rate by respondents to the questionnaire according to the five age periods indicates that infants (≤ 1yr) in households served by public water supply consumed about 5 glasses of tap-water per day, equivalent to 1000ml or 1 L per day while those on PWS consumed about 4.5 glasses (900ml or 0.90 L/day). For children 1-6 years served by public water, intake rate was 5 glasses (1000ml or 1 L) while those on PWS consumed about 5.5 glasses (1100ml or 1.10 L). Children 7-12 years on public supply consumed 5.5 glasses (1100ml or 1.10 L) while those on PWS consumed 5 glasses (1000ml or 1 L). The rate of consumption for adolescents (13-18 years) and adults (19-70 years) served by public supply were 3.5 mugs

(875ml or 0.875 L/day) and 4-5 mugs (11250ml or 1.125 L/day) respectively. For those on PWS the rate was 3 mugs (750 ml or 0.75 L/day) and 4-6 mugs (1250 ml or 1.25 L/day) respectively. On average, the intake rate for infants was 0.95 L/day; child 1.05 L/day; child 1.0 L/day; adolescent 0.8 L/day and adults 1.2 L/day.

Although infants (≤1yr) and children (7-12 years) served by public supply consumed slightly more water than their counterparts served by PWS, and whereas child (1-6yrs) and adults served by PWS consumed slightly more water than their counterparts on public supply, a T-test statistical analysis (Table 5) shows that there was no statistically significant difference in tap-water intake between public water supply and PWS users. Table 6 shows the parameters and assumption used in calculating the CDI. Given that individual body weight changes over a lifetime (USEPA,1989b; Covello & Merkhofer,1993), and following USEPA exposure assessment guidelines (USEPA, 1997), average body weights of 9.5kg; 15kg; 28kg; 53kg and 65kg for infants; children (1-6 years); children (7-12 years); adolescents and adults respectively for both male and female were used to calculate CDI.

Table 6: Parameters and Assumption for Calculating CDI.

Parameters	**Infant (≤1yr)**	**Child (1-6yrs)**	**Child (7-12yrs)**	**Adolescent (13-18yrs)**	**Adult (19-70yrs)**
Contact Rate (CR)	0.95 L	1.05 L	1.00 L	0.80 L	1.20 L
Exposure Frequency (CF)	365days	365days	365days	365 days	365 days
Exposure Duration (ED)	1yr	5yrs	6yrs	6yrs	52yrs
Body Weight (BW)	9.5 kg	15 kg	28kg	53kg	65kg
Averaging Time (AT)	1yrs	5yrs	6yrs	6yrs	52yrs

Exposure duration (ED) is usually over a lifetime (70 years) by convention for carcinogens (USEPA, 1989b; Gerba, 1996). Given that tap-water intake changes with age, body weight, diet and climate (Gerba, 1996), ED was divided according to the five age periods (1 year for infants; 5 years for children 1-6years; ; 6 years for children 7-12 years; 6 years for adolescents and 52 years for adults) to reflect lifetime exposure. For Averaging Time (AT), the default assumption for carcinogens is 70 years (USEPA, 1989) and this was also divided according to the five age periods to reflect a lifetime. The exposure frequency (EF) is number of days in a year (365 days per year). The CDI for each of the five age periods was calculated by substituting the figures in table 6 in equation 2 and then summing as in Equation 3 to give the total CDI for each of the exposure categories as presented in Table 7.

Equation 3:

CDI =

$$\frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ infant\ (\leq 1yr) + \frac{Cx\ CR\ xEFxED}{BW\ x\ AT}\ (child\ 1-6\ yrs) + \frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ (child\ 7-12\ yrs)$$

$$+\frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ (adolescent\ 13-18yrs) + \frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ (adult\ 19-70yrs)$$

Table 7: Chronic Daily Intake (CDI) of Nitrate in drinking water by Age Group

Nitrate level (mg/l)	Mean Level (C)	CDI (mg/kg/day)					
		≤1yr	1-6yrs	7-12yrs	13-18yrs	19-70yrs	Total
0-5	2.5	0.25	0.20	0.09	0.04	0.05	0.63
6-10	8	0.80	0.56	0.30	0.12	0.15	1.93
11-15	13	1.30	0.91	0.46	0.19	0.24	3.10
16-20	18	1.80	1.26	0.64	0.27	0.33	4.30
21-25	23	1.90	1.61	0.82	0.35	0.42	5.10
26-30	28	2.80	1.96	1.00	0.40	0.52	6.68
31-35	33	3.30	2.31	1.18	0.50	0.61	7.90
36-40	38	3.80	2.66	1.36	0.60	0.70	9.12
41-45	43	4.30	3.01	1.53	0.65	0.79	10.28
46-50	48	4.80	3.36	1.71	0.72	0.89	11.48
51-55	53	5.30	3.71	1.90	0.80	0.97	12.68
56-60	58	5.80	4.06	2.07	0.87	1.10	13.90
61-65	63	6.30	4.41	2.25	0.95	1.20	15.11
66-70	68	6.80	4.76	2.43	1.03	1.25	16.27
71-75	73	7.30	5.11	2.61	1.10	1.35	17.47

Table 7 (Continued): Chronic Daily Intake (CDI) of Nitrate in drinking water by Age Group.

Nitrate level (mg/l)	Mean Level (C)	CDI (mg/kg/day)					
		≤1yr	1-6yrs	7-12yrs	13-18yrs	19-70yrs	Total
76-80	78	7.80	5.46	2.78	1.18	1.44	18.66
81-85	83	8.30	5.81	2.96	1.25	1.50	19.82
86-90	88	8.80	6.16	3.14	1.33	1.62	21.05
91-95	93	9.30	6.51	3.32	1.40	1.70	22.23
96-100	98	9.80	6.86	3.50	1.48	1.80	23.44
101-105	103	10.30	7.20	3.68	1.55	1.90	24.63
106-110	108	10.80	7.56	3.86	1.63	1.99	25.84
111-115	113	11.30	7.91	4.03	1.70	2.10	27.04
116-120	118	11.80	8.26	4.21	1.78	2.17	28.22
121-125	123	12.30	8.61	4.39	1.86	2.30	29.46
126-130	128	12.80	8.96	4.57	1.93	2.36	30.62
131-135	133	13.30	9.31	4.75	2.00	2.45	31.81
136-140	138	13.80	9.66	5.00	2.08	2.50	33.04
141-145	143	14.30	10.01	5.11	2.15	2.60	34.17
146-150	148	14.80	10.36	5.28	2.23	2.70	35.37
151-155	153	15.30	10.71	5.46	2.31	2.80	36.58
156-160	158	15.80	11.06	5.64	2.38	2.90	37.78
161-165	163	16.30	11.41	5.82	2.46	3.00	38.99
166-170	168	16.80	11.76	6.00	2.53	3.10	40.19
171-175	173	-	-	-	-	-	-
176-180	178	17.80	12.46	6.36	2.70	3.30	42.62
181-185	183	18.30	12.81	6.53	2.76	3.40	43.80
186-190	188	18.80	13.16	6.71	2.84	3.50	45.01
191-195	193	-	-	-	-	-	-
196-200	198	19.80	13.86	7.07	3.00	3.65	47.38
201-205	203	20.30	14.21	7.25	3.06	3.70	48.52

Table 7 (Continued): Chronic Daily Intake (CDI) of Nitrate in drinking water by Age Group.

Nitrate level (mg/l)	Mean Level (C)	CDI (mg/kg/day)					
		≤1yr	1-6yrs	7-12yrs	13-18yrs	19-70yrs	Total
206-210	208	20.80	14.56	7.43	3.14	3.80	49.73
211-215	213	21.30	14.91	7.61	3.21	3.90	50.93
216-220	218	21.80	15.26	7.78	3.30	4.00	52.14
221-225	223	-	-	-	-	-	-
226-230	228	22.80	15.96	8.14	3.44	4.20	54.54
231-235	233	23.30	16.31	8.15	3.52	4.30	55.58
236-240	238	23.80	16.60	8.50	3.59	4.40	56.89
241-245	243	-	-	-	-	-	-
246-250	248	24.80	17.36	8.86	3.74	4.60	59.36
276-280	278	27.80	19.46	10.00	4.20	5.10	66.56
281-285	283	-	-	-	-	-	-
286-290	288	28.80	20.16	10.29	4.45	5.30	69.00
316-320	318	31.80	22.26	11.36	4.80	5.90	76.12
376-380	378	37.80	26.46	13.50	5.71	6.97	90.44
386-390	388	38.80	27.16	13.86	5.86	7.20	92.88
416-420	418	41.80	29.26	14.93	6.31	7.70	100.00
431-435	433	43.30	30.31	15.46	6.53	8.00	103.60
466-470	468	46.80	32.76	16.71	7.06	8.64	111.97

Abbreviation:

CDI = chronic daily intake

Mg/kg/day = milligram per kilogram per day

Yrs = years

The CDI is incorporated into Equation 1 in the risk characterisation stage to estimate the unit risk of goitre in each nitrate exposure category.

6.3 DISCUSSION

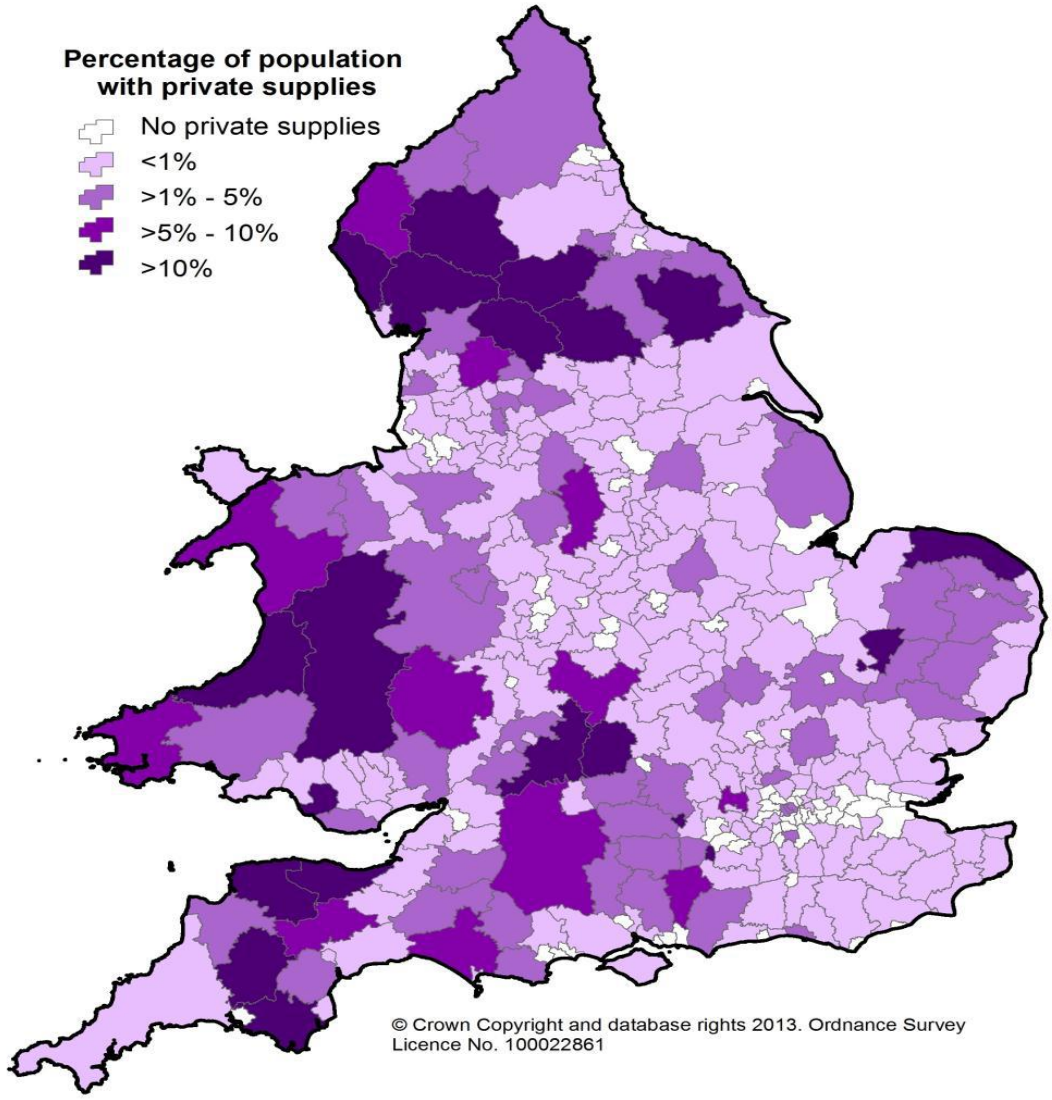
Analysis of nitrate data from public water and PWS in East Anglia shows that higher nitrate levels are encountered in PWS than in public water supplies. The high nitrate levels in PWS may be due to their susceptibility to pollution as the boreholes or wells are usually shallower when compared to public supply boreholes which are usually located in deep chalk and therefore not readily affected by pollution or weather patterns (DWI, 2013). Although nitrate concentration was higher in PWS than in public supply, the number of people served by PWS and therefore exposed to high nitrate concentrations as captured in this exposure assessment were far less than the number of people served by public water (see Table 6). In England, whilst there are about 44,546 PWS locations (52 per cent of UK total), the number of people served is 872,746 or 1.6 per cent of the total population of 53,012,456 (DWI, 2013). Although South West England has a higher number of PWS (15,309) compared to East of England including East Anglia (5,285), the population served by PWS was higher (380,652) than that of South West England (58,256) (DWI, 2013). This suggests that the population in East of England, including East Anglia is more exposed to water from private sources in England. Table 8 shows the number of PWS and the population served per regions in England.

Table 8 Number of PWS per region in England (DWI, 2014)

Region	**No of PWS**	**No. of people served**
East Midlands	1,581	39,569
West Midlands	6,595	32,032
East of England	5,285	380,652
North East England	1,891	5,334
North West England	6,144	104,437
Yorkshire/Humberside	5,051	121,538
London & South East	2,690	130,928
South West England	15,309	58,256
Total	**44,546**	**872,746**

According to DWI (2014), there are about 77 Local Authorities in England where more than 1per cent of the resident population only relies on PWS for their drinking water. In Cumbria, Yorkshire, East Anglia and parts of Devon, DWI (2014) reported that more than 5 per cent of the resident population relies on PWS for their drinking water and therefore recommended that, "the safety of PWS should feature as an explicit component of the Local Authority's public health protection strategy" (DWI 2014 pg. 8). They also recommended that such Local Authorities "use the population-based risk information of the Inspectorate to ensure that the risk management of PWS is prioritised within the Authority's health protection strategy" and that "all local health protection strategies should in future reference the local figure for access to a reliable supply of water" (ibid, pp. 8 & 9). Figure 18 shows areas in England (including East Anglia) where more than one per cent of the population do not have access to water from public supply and therefore rely on PWS.

Figure 18:



Although nitrate concentrations in PWS are higher than in public water, a survey of the drinking water habits of the population in Suffolk Coastal area revealed that in households served by PWS, no child in the age group 0-5 years was served with water from PWS. Whilst the reason for this is not clear, a possible explanation may be due to the Advice Notes (Appendix 8) issued by the Council's Environmental Health Officers, the Health Protection Agency (now Public

Health England) and midwives to expectant mothers in households served by private water supplies on the association between nitrates in drinking water and infantile methaemoglobinemia; and the need to use alternative sources of drinking water especially in preparing infant formula if nitrates are found to be above 50mg/l in PWS. This may explain why bottled water usage was higher by infants in households served by PWS than in those served by public water. Although three women aged 31to 40 years served by PWS also used bottled water, it was not certain if these women were pregnant at the time of this survey or preferred bottled water as this information was not collected. Although there is no statistically significant difference in tap-water consumption between public water and PWS users ($p>0.05$), on average, infants and children ≤1 to 6yrs had a higher tap-water intake per day than adolescents (0.8L). The reasons for this may be because of the fluid based nature of the infant diet especially those aged 0-2years and the amount of water used in reconstituting infant's formula. Whilst the majority of tap-water intake by adults was associated with hot drinks e.g. coffee, tea, and hot chocolate, the fact that this survey was conducted during the autumn and winter months may have influenced the amount of tap-water intake for hot drinks. Although the majority of the children's tap- water intake was associated with squash drinks, their low tap-water intake when compared to the adults may be because of their preference for fizzy drinks or drinking away from home (e.g. school).

The amount of tap-water consumed in this population (0.8 - 1.2L/day) compares with the average daily water consumption of 0.10L - 1.55L/day reported by Mons et al (2007). Also, it compares with the levels of water intake reported in the rest of the UK population where an average consumer has been reported to consume about 1.14 L tap-water per day (DWI 1996); 0.70 - 1.9L/day (Hunter et al, 2004); 0.9-1.3L/day (Hopkin & Ellis; 1980). In Canada, EHD (1981) reported tap-water consumption rate of 0.57-0.66 L/day for children aged <3years;

0.88L/day for 3-5 years old and 1.14L/day for 6-17 years old children. The amount of tap-water intake in the USA for children aged 11-19 years was 0.44L/day (USEPA, 2000).

Although the amount of tap-water consumption in the population compares with the rest of the UK population and other countries, several factors are known to influence water consumption. They include diet, regional climate, temperature, season, age, bodyweight, gender, physical activity, cultural differences, and employment status (Haring et al, 1979; Hopkin & Ellis, 1980; EHD, 1981; Gerba, 1996; Shimokura et al, 1998; Kaur et al, 2004). Whilst the influence of each of these factors on water consumption may be contradictory (Mons et al, 2007), higher water consumption has been reported among people who are involved in heavy physical work than those who are not extremely active during their work (Toth et al, 1977; EHD, 1981). Although tap-water intake outside the home was not considered in this survey, available evidence suggest that 63-67 per cent of all tap-water intake take places at home (Shimokura et al, 1998; Kaur et al, 2004). The influence of gender and employment status on tap-water intake was not an objective of this survey and therefore was not considered. However, available evidence suggests that employment status has a stronger influence on tap-water intake than gender (Shimokura et al, 1998). While women who are employed part-time or unemployed are reported to consume more tap-water (probably for hot drinks) at home than men, employed men are reported to consume more tap-water outside the home, the majority of which are for hot drinks (Shimokura et al, 1998). According to EHD (1981), people who were extremely active during their work or in their spare time consumed 1.72L and 1.57L/day respectively while those who were not active during their work or in their spare time consumed 1.30L and 1.35L/day respectively.

Analysis of the chronic daily intake (CDI) of nitrates in drinking water shows that nitrate body burden increases with increasing concentration in drinking water (See Table 7). It also shows that nitrate body burden was higher in infants (≤1year); and children (1-6years) and (7-12 years) than in adolescents and adults even when they consumed the same or lesser amount of water. For example, at the drinking water standard of 50mg/l NO_3^-, nitrate body burden for infants ≤1year was calculated as 4.8 mg/kg/day; 3.36kg/mg/day for a child 1-6years; 1.71mg/kg/day for a child 7-12years; 0.72 mg/kg/day for adolescents and 0.89mg/kg/day for adults (19-70yrs). Also at 100mg/l, the body burden for infants; children (1-6 years); children (7-12 years); adolescent and adults are 9.8; 6.86; 3.50; 1.48 and 1.8mg/kg/day respectively. These figures suggest that nitrate body burden is more on infants and children 1-6years is more than five times that of adolescents and adults and twice that of children 7-12 years and this further suggests that infants and children are more vulnerable to nitrate contaminated drinking water than adolescents and adults. The reason for this may be because of the small body weight of infants and children relative to that of adolescents and adults.

6.3.1 RISK ASSESSMENT BASED LEGISLATION FOR PRIVATE WATER SUPPLIES

The concept of risk assessment has long existed in various forms and has been used to drive legislation for public health protection (NRC, 1983, 2008; Ritter et al, 2002; Ball, 2006). Under the WHO Guidelines for Drinking Water Quality, risk assessment is central in the Water Safety Plan (designed for safeguarding the health of the water the consumers) (WHO, 2004) and has been used to assess the microbial and chemical safety of drinking water quality (Mons et al, 2007). However, the EU Directive 98/83/EC on drinking water quality does not require risk

assessment and therefore this did not drive its inclusion in the UK PWS Regulation (2009). Its inclusion in this piece of legislation, according to DEFRA, was driven by the WHO Water Safety Plan and the methodology developed by the drinking water regulator in Scotland (personal communication). The WHO Water Safety Plan which aims to provide a comprehensive risk-based approach to drinking water quality management requires the development of a thorough understanding of the supply process, from source to tap, and the implementation of measures to identify and mitigate the risk associated with contamination; reduced inefficiency and/or failure of treatment; deterioration of quality or contamination in distribution on consumer premises (Chartered Institution of Water and Environmental Management (CIWEM), 2004; WHO, 2009).

The Private Water Supply Regulation (2009) which came into force in England &Wales in January 1, 2010 requires Local Authorities to within 5 years; carry out a risk assessment of all large and small PWS (except supplies serving single dwellings) in their area to determine if it poses a potential danger to human health and if so, to take action to safeguard public health in the short term and to improve the supply in the long term. Although the purpose of the PWS Regulation is to create a national record of all PWS to enable effective public health protection, and to enable the UK government provide data to the EU Commission in compliance with the Drinking Water Directive (DWI, 2013), DEFRA consider the risk assessment based approach in the legislation as a means of overcoming the limitations of sampling PWS at very low frequencies as was the case in the earlier version of the PWS Regulation (Personal Communication). Also it is a means of overcoming the problems that are sometimes encountered by Local Authorities where a PWS may be found to comply with the regulation in all parameters in dry weather but fail to comply after a period of rain (Personal Communication).

In compliance with the regulation, and by 2012, about 96% of all local authorities in England & Wales had provided information on their PWS (name, location, size, purpose, type) for the purposes of the national records, progress on the risk assessment side of the regulation has not been impressive (DWI, 2013). According to the DWI (2013), only 17 per cent of all PWS in England and 27 per cent in Wales had been risk assessed by the end of 2012. The majority of the PWS so far risk assessed were however commercial supplies or large supplies serving food premises (48 per cent); public buildings (48 per cent); tourism and leisure centres (39 per cent). These figures compare favourably with the 2011 figures i.e. food premises (36 per cent), public buildings (26 per cent), tourism and leisure (22 per cent). Although it is not known if this risk assessment based legislation will lead to improvements in the quality of the large and small PWS in the long run, available evidence however suggest that slight improvements have been recorded in these supplies (DWI, 2013). For example in 2012, 7.5 per cent of tests failed to meet the drinking water standards compared to 9.6 per cent test failures in 2010. Also in 2012, local authorities served 412 Improvement or Restriction Notices on PWS identified as posing potential danger to human health. This is more than the 237 such notices served in 2011 (DWI 2013). In addition, 17 notices were served in 2012 for either insufficient or unwholesome PWS as against 15 of such notices served in 2011 (DWI, 2013).

Whilst the EU Directive 98/83/EC applies to large supplies i.e. all private water supplies providing water of $10m^3$/day or more (serving 50 or more persons) and /or part of a commercial or public activity (such as bed & breakfast establishments), it does not apply to small supplies (supplies providing water of less than $10m^3$/day or serving less than 50 persons) unless they are part of a commercial or public activity. But in implementing the Directive through the Private Water Supplies Regulation 2009, the UK government thought it wise to apply this Directive to small supplies (except supplies that serve single dwellings) on the argument that, “the people

consuming water and food prepared with water derived from these small supplies are entitled to the same level of health protection as the people served by large supplies and public water" (DWI 2010, p.8). But given that 58 per cent of the 44,546 PWS in England serve single dwellings, the exclusion of these supplies from the 5 years mandatory risk assessment and monitoring regime as provided in the legislation could put the health of people (especially infants and children) living in such single dwellings at risk of nitrates and other drinking water contaminants. Although the Regulations require Local Authorities to record the locations of this type of supply, they are not required to carry out risk assessment and monitoring unless requested to do so by the owner or user, or if the supply comes to the attention of the Environmental Health Officer for some other reason, such as change of ownership or use.

Whilst the Regulation may in the long term lead to improvements in the quality of large and small PWS, it is not yet clear how it could lead to improvements in those small supplies serving single dwellings given the non-mandatory risk assessment and monitoring regime as it applies to these supplies. Although it is not clear why the regulation excluded this type of supply from the mandatory risk assessment and monitoring regime, a possible explanation may be along the lines of the view expressed by Reacher et al (1999), that this type of supply is not large enough to pose a substantial risk to public health. However, given that about 60,000 people in England alone live in 25,956 single domestic dwelling served by PWS (DWI 2013), the exclusion of these supplies from the mandatory risk assessment could put the health of people living in such dwellings at risk of nitrate pollution, thus leaving this area of public health still determined more by local policy rather than legislation.

6.4 CONCLUSION

Nitrate contamination of drinking water is widespread in the UK, including East Anglia. Although the quality of public water has greatly improved in the UK in recent years, the same could not be said of PWS. Higher nitrate levels had been found in PWS than public water, but the number of people regularly exposed to PWS in their homes is far less than the number of people exposed to public water. However, many more people are exposed to high nitrate levels in PWS in their places of work or business, or while attending leisure events; staying in hotels; or bed & breakfast accommodations served by PWS. Although South West England has the highest number of PWS compared to other regions of England, more people are exposed to PWS in east of England including East Anglia than in other regions. Whilst there is no statistically significant difference in tap-water consumption between PWS and public water users, the average amount of tap-water consumed in the study population was 0.95 L (infants ≤ 1year); 1.05 L (child, 1-6 years); 1.00 L (child, 7-12 years); 0.8 L (adolescent 13-18years) and 1.2 L (adults, 19-70 years). Nitrate body burden was found to be higher in infants and children than in adolescents and adults even when they drank the same or lesser amount of water. This suggests that infants and children may be more vulnerable to nitrate contaminated drinking water. The high nitrate body burden on infants and children may be due to their small body weight relative to the adults.

Whilst the new PWS Regulation may in the long term lead to improvement in large and small PWS, it is not clear whether such improvement could be achieved in the small supplies that serve single dwellings which are currently exempt from the 5 years mandatory risk assessment regime. Given that the responsibility for the safety and quality of this type of supply lies with the owner

or user, and that risk assessment and monitoring is at the discretion of the Local Authority, achieving the desired improvement in water quality may be difficult. This is because in the current economic climate where every Local Authority is plagued by resource issues, they may not have the capacity to risk assess this type of supply even when requested to do so by the owner or user. Another important factor that may stand in the way of intervention in this area of public health is the attitude of the people who own these supplies. It is usually difficult to convince PWS owners of the need to install an appropriate treatment system even given that the PWS could easily be contaminated. Also, it can be very difficult to persuade people that there is a contamination problem in a supply that presents a potential health risk when the owner explains "they have drank the water all their lives and have never been ill". Even serving an Improvement Notice where such supplies are identified as posing a potential danger to human health or the quality of water is unwholesome may be seen as 'intrusion by the state'.

Given that the majority of the PWS in East Anglia (and other regions of the UK) serve single dwellings and the fact that this type of supply is more susceptible to microbial and chemical contamination, the government could extend the mandatory risk assessment and monitoring regime to these small supplies on the same argument that people consuming water and food prepared with water derived from these small supplies are entitled to the same level of health protection as the people served by large PWS and public water. This will ensure the wholesomeness of water from these supplies and also protect the health of the people served by these supplies from chemical and microbial contaminated drinking water.

CHAPTER SEVEN

7.0 RISK CHARACTERISATION

7.1 INTRODUCTION

Risk characterisation is the last stage of the risk assessment process and involves integrating information from dose-response analysis and exposure assessment in order to estimate excess risk of a disease risk factor in individuals or a population (Robert & Abernathy 1996; Ball 2006). Also, it involves the characterisation of the strengths, limitations and uncertainties inherent in the risk estimates. It provides information that can be used by policy makers to inform risk management or regulatory options (Robert & Abernathy 1996; Ball 2006), taking into consideration social, political and economic issues as well as engineering problems inherent in any proposed solution (Gerba, 1996). The results of risk characterisation can be used to target prevention and remediation or control efforts in areas, sources or situations where the greatest risk reduction can be achieved with the available resources (Gerba, 1996).

The purpose of this chapter therefore is:

- To estimate the excess risk of thyroid hypertrophy (goitre) in East Anglia as a result of exposure to nitrates in drinking water by integrating information from dose - response analysis and exposure assessment.
- To characterise the strength and limitations of the study.
- To characterise uncertainties associated with the risk estimates.

- To evaluate the appropriateness of the current nitrate drinking water standard of 50mg/l (set to protect against infantile methaemoglobinemia) in protecting against thyroid hypertrophy, taking into account nitrate exposure from other sources.

The concept of excess risk or attributable fraction (AF) was first proposed by Levin in 1953 (Levin, 1953). It is defined as the proportion of disease cases in a population over a specified period of time that can be attributed to a risk factor (Levin, 1953; Hennekens & Buring, 1987; Rockhill et al, 1998; Rosen, 2013); assuming exposure to the risk factor is causally related to the health outcome (Rockhill et al, 1998). In other words, it is the proportion of disease cases in a population that can be prevented if exposure to the risk factor is eliminated (Hennekens & Buring, 1987; Rockhill et al, 1998), while distributions of other risk factors (to the health outcome) in the population remain unchanged (Rockhill et al, 1998). The term attributable fraction is sometimes referred to as population attributable fraction (PAF); population attributable risk (PAR); population attributable risk proportion; excess risk or fraction; etiological fraction and incidence density fraction (IDF) (Rockhill et al, 1998; US Department of Health and Human Services (USDHHS), 2004; Rosen, 2013), however, the terms PAF or excess risk are more appropriate (Greenland & Robin, 1988; Rockhill et al, 1998) and therefore preferred in this study.

PAF combines relative risk and the prevalence of exposure to measure the public health burden of a risk factor by estimating the proportion of cases of a disease that would not have occurred if exposure was eliminated and is widely used in public health policy to set priorities for action and in planning public health interventions (Nothridge, 1995; Rowe et al, 2004; Steenland & Armstrong, 2006). It has been used to estimate the number and or the percentage of cancers attributable to lifestyle and environmental factors, including tobacco smoking (Parkin, 2011a &

b) and alcohol consumption in the UK (Parkin, 2011c). Also, it has been used to quantify the health damage attributable to tobacco use (Rosen, 2013); occupational exposures (Steenland & Armstrong, 2006) and the health benefits or risk of physical activity (Macera & Powell, 2001).

The World Health Organisation in 2003 published an introduction to the methodology for assessing the environmental burden of diseases (EBD) (WHO, 2003) as part of their global burden of disease (GBD) work (Murray & Lopez, 1996). The report gave the background to and a description of the general methods developed for quantifying the health impact (whether disease, injury or other health conditions) attributable to a particular environmental risk at a population level. This methodology which is essentially based on calculating the PAF aims to provide a means to help policy makers prioritise public health policies and actions directed at preventing or reducing the health impact of environmental risks; identifying high-risk groups in the population and also estimating the health gains intervention can bring (Ormandy & Braubach, 2011). It also aims to raise awareness and strengthen institutional capacity for reducing the impact of environmental health risks on the population (WHO, 2003). This methodology has been used in quantifying the health impact of some housing risks, such as indoor radon and lung cancer (Zeeb, 2011) and household crowding and tuberculosis (Baker et al, 2011) in Europe. Although various methods can be used in quantifying the burden of disease attributable to specific exposures or risk factors, while the majority of these methods have been based on decades of observation of the exposed group and mathematical models (United States Department of Health & Human Sciences (USDHHS), 2004; Rosen, 2013), calculating the PAF or excess risk is a more popular method and is widely used (WHO, 2003; USDHHS, 2004).

7.2 INTEGRATING DOSE-RESPONSE AND EXPOSURE ASSESSMENTS

7.2.1 DOSE-RESPONSE ANALYSIS

Equation 1 (first outlined in section 5.3) combines the slope of the regression from dose – response assessment with the chronic daily intake (CDI) of nitrates in drinking water (equation 3, section 6.2) in order to determine the relative risk (RR) of thyroid hypertrophy (goitre) in each exposure category.

$y = mx+1$ **Equation 1**

Where:

y = Relative Risk (RR)

m = slope of the dose-response (slope factor) = 0.0347mg/kg-bw/day.

x = chronic daily intake (CDI) of nitrates in drinking water in East Anglia (exposure assessment).

1 = a parameter that describes the background risk.

7.2.2 EXPOSURE ASSESSMENT

The CDI was calculated as in Equation 3 (section 6.2) for each age group and summed to represent CDI over a life time of 70 years.

(Equation 3)

CDI =

$$\frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ \boldsymbol{infant}\ (\leq \mathbf{1}\boldsymbol{yr}) + \frac{C\ x\ CR\ xEFxED}{BW\ x\ AT}\ (\boldsymbol{child}\ \mathbf{1-6}\ \boldsymbol{yrs}) + \frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ (\boldsymbol{child}\ \mathbf{7-12}\ \boldsymbol{yrs})$$

$$+ \frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ (\boldsymbol{adolescent}\ \mathbf{13-18}\boldsymbol{yrs}) + \frac{C\ x\ CR\ x\ EF\ x\ ED}{BW\ x\ AT}\ (\boldsymbol{adult}\ \mathbf{19-70}\boldsymbol{yrs})$$

Given that there is no register for goitre (benign thyroid tumour) in the UK, it was not possible to estimate the incidence of benign thyroid tumour in the population and as a result, thyroid cancer was used as a proxy for goitre given that thyroid cancer incidence is well reported in the UK. There is a close link between benign and malignant tumours (Leux & Guenel, 2010). According to Franceschi et al (1999), the existence of goitre or nodule of the thyroid is associated with excess risk of thyroid cancer. Some non-cancerous (benign) conditions of the thyroid such as nodules (adenomas); enlarged thyroid (goitre); and inflammation of the thyroid (thyroiditis) can increase the risk of thyroid cancer (Cancer Research UK, 2014). Benign thyroid tumours may share common aetiological factors with malignant tumours and can be seen as precancerous lesion (Leux & Guenel, 2010). According to Rahman et al (2010) 13 or 8.2 per cent of 160 goitre cases reported in a hospital in Nigeria were malignant. Also in Kano State, Nigeria, 24 or 15 per cent of 160 goitre cases were cancerous (Edino et al, 2010).

In order to calculate the excess risk or PAF for thyroid cancer, information on thyroid cancer incidence in East Anglia was obtained from the Office of National Statistics (ONS). In this context, PAF represents the proportion of thyroid cancer cases in East Anglia that can be attributed to exposure to nitrates in drinking water and thus could be prevented if the exposures were eliminated; assuming that exposure to nitrates in drinking water is causally related to

thyroid cancer. Given that the latent period or the interval between exposure and development of cancer is not known, it was assumed that this would be 10 years or more (Parkin 2011a) and thus, the number of thyroid cancer cases expected in East Anglia in 2014 was calculated, having obtained data on nitrate concentrations in drinking water from 2001 to 2010 as already stated in section 4.5. Cancer incidence statistics for 2014 is the latest data published by ONS (2016).

Calculating PAF therefore relied on the following information:

- Dose-response relationship - The slope factor (q_1^*) 0.0347mg/kg-bw/day, derived in section 5.3.
- Exposure assessment - The concentration of nitrates in drinking water in East Anglia, the population served and the chronic daily intake (CDI) of nitrates derived in section 6.2.
- The crude incidence rate for thyroid cancer in England in 2014 - obtained from the Office of National Statistics (ONS). The crude incidence rate is the simplest method of comparing cases or deaths accounting for population size only (Cancer Research, UK, 2016).
- Data on age-group thyroid cancer incidence obtained from the Office of National Statistics (ONS).

The first step in PAF calculation involved deriving the relative risk of thyroid cancer for each nitrate exposure category. This was done by integrating the slope factor (q_1^*) and the CDI (x) using the linear non-threshold (LNT) model (Equation 1) as already described in Section 5.3,pg124.

Given that the PAF does not depend entirely on the relative risk (RR) due to exposure but also on the fraction of the population exposed and the prevalence of the risk factor in the population (Steenland & Armstrong, 2006), the general formula for PAF is as shown in equation 4.

Equation 4

$$\boldsymbol{PAF = \frac{p(RR-1)}{p(RR-1)+1}}$$

Where:

p = proportion of the population exposed to nitrates in drinking water.

RR = the risk of thyroid cancer.

Given that the entire population in East Anglia is exposed to nitrates in drinking water (albeit at different concentrations), this reduces the formula to equation 5 and this was used in this study to, first, calculate the AF.

Equation 5

$$AF = \frac{RR_c - 1}{RR_c}$$

Where:

RR_c is the (adjusted) relative risk for each exposure category - (C)

Equation 5 is used in PAF calculation because it accounts for uncertainties inherent in cause and effect relationships and therefore produces internally valid estimates of AF unlike equation 4

which is not valid in the presence of uncertainties (Miettinen, 1974; Rockhill et al, 1998). Also, it is used when there is partitioning or categorisation of exposure in the population and the use of adjusted relative risks in calculating AF (Rockhill et al, 1998) as was the case in this study. As noted by Rockhill et al (1998), PAF can be quantitatively partitioned or distributed into exposure – category - specific attributable fraction and then summed as the overall PAF. The exposure – category - specific attributable fraction termed the 'distributive property' (Miettinen, 1974; Wacholder et al, 1994) is defined as, "the fraction of total disease risk in the population that would be eliminated if persons in only that specific- exposure- category were to be shifted to the unexposed group" (Rockhill et al, 1998:pg16).

Thyroid cancer cases in East Anglia was calculated by dividing the number of people in each exposure category by 100,000 and then multiplying by the crude rate of thyroid cancer for 2014. The crude rate of thyroid cancer in 2014 was 5.4 cases per 100,000 (Cancer Research UK, 2014). The PAF for each exposure category was obtained by multiplying the number of thyroid cancer cases by the AF in that exposure category and then summing to give the overall PAF.

PAF can be expressed as a percentage using equation 6.

$$PAF\% = \frac{PAF}{No\ of\ cases} \times 100 \qquad \textbf{Equation 6}$$

7.3 RESULT

Table 9 shows the risk estimates of thyroid cancer in East Anglia. It shows for each exposure category the mean nitrate concentration in drinking water in East Anglia; the CDI of nitrates over a lifetime by individuals in the population; the relative risk of thyroid cancer and the excess risk

(AF). Also, it shows the population in each exposure category as obtained from water companies and Local Authorities; the number of thyroid cancer cases calculated for a population of 2,849,918 in East Anglia and the corresponding PAF due to chronic exposure to nitrates in drinking between 2001 and 2010. The crude rate for thyroid cancer (5.4 cases per 100,000) for 2014 was used in the calculation of PAF because that is the latest thyroid cancer statistics published by the Office of National Statistics (ONS, 2016; Cancer Research UK, 2016).

Table 9: Thyroid Cancer Risk Estimates.

Nitrate level (mg/l)	Mean level (mg/l) (C)	CDI (mg/kg-bw/ day) (X)	RR= mx+1	AF $\frac{RR-1}{RR}$	Population (P)	No of Cases of thyroid cancer $\frac{p}{100{,}000} \times 5.4$	PAF= AF x No of cases.	PAF %= $\frac{PAF}{No\ of\ cases} x100$
0-5	2.5	0.63	1.02	0.02	680,638	36.75	0.74	2
6-10	8	1.93	1.07	0.06	141,636	7.65	0.46	6
11-15	13	3.10	1.10	0.09	517,546	27.95	2.50	9
16-20	18	4.30	1.14	0.12	150,578	8.13	0.97	12
21-25	23	5.10	1.18	0.15	150,454	8.12	1.22	15
26-30	28	6.68	1.23	0.18	301,845	16.30	2.93	18
31-35	33	7.90	1.27	0.21	367,132	19.82	4.16	21
36-40	38	9.12	1.32	0.24	464,704	25.09	6.02	24
41-45	43	10.28	1.36	0.26	47,673	2.574	0.67	26
46-50	**48**	**11.48**	**1.40**	**0.28**	**23,574**	**1.273**	**0.36**	**28**
51-55	53	12.68	1.43	0.30	540	0.029	$8.7x10^{-3}$	30
56-60	58	13.90	1.48	0.34	336	0.018	$6.1x10^{-3}$	34
61-65	63	15.11	1.52	0.34	330	0.017	$5.8x10^{-3}$	34
66-70	**68**	**16.27**	**1.56**	**0.38**	**210**	**0.011**	$\mathbf{4.2x10^{-3}}$	**38**
71-75	73	17.47	1.60	0.39	108	$5.8x10^{-3}$	$2.27x10^{-3}$	39
76-80	78	18.66	1.65	0.41	246	0.013	$5.4x10^{-3}$	41
81-85	83	19.82	1.69	0.42	174	$9.4x10^{-3}$	$3.95x10^{-3}$	42
86-90	88	21.05	1.73	0.43	138	$7.4x10^{-3}$	$3.2x10^{-3}$	43
91-95	**93**	**22.23**	**1.77**	**0.45**	**174**	$\mathbf{9.4x10^{-3}}$	$\mathbf{4.22x10^{-3}}$	**45**
96-100	98	23.44	1.81	0.46	312	0.017	$7.8x10^{-3}$	46
101-105	103	24.63	1.85	0.47	354	0.019	$8.9x10^{-3}$	47
106-110	108	25.84	1.90	0.48	258	0.014	$6.7x10^{-3}$	48
111-115	113	27.04	1.94	0.49	54	$2.9x10^{-3}$	$1.43x10^{-3}$	49
116-120	118	28.22	1.98	0.50	114	$6.1x10^{-3}$	$3.08x10^{-3}$	50
121-125	123	29.46	2.02	0.51	42	$2.3x10^{-3}$	$1.17x10^{-3}$	51
126-130	128	30.62	2.06	0.52	66	$3.6x10^{-3}$	$1.87x10^{-3}$	52
131-135	133	31.81	2.10	0.53	42	$2.3x10^{-3}$	$1.22x10^{-3}$	53
136-140	138	33.04	2.15	0.54	72	$3.9x10^{-3}$	$2.11x10^{-3}$	54
141-145	143	34.17	2.18	0.55	54	$2.9x10^{-3}$	$1.6x10^{-3}$	55
146-150	148	35.37	2.33	0.56	102	$5.5x10^{-3}$	$3.1x10^{-3}$	56
151-155	153	36.58	2.27	0.57	18	$9.7x10^{-4}$	$5.53x10^{-4}$	57
156-160	158	37.78	2.31	0.57	42	$2.3x10^{-3}$	$1.31x10^{-3}$	57
161-165	163	38.99	2.35	0.57	12	$6.5x10^{-4}$	$3.71x10^{-4}$	57

Table 9 (Continued): Thyroid Cancer Risk Estimates.

Nitrate level (mg/l)	Mean level (mg/l) (C)	CDI (mg/kg-bw/ day) (X	RR mx+1	AF $\frac{RR-1}{RR}$	Population (P)	No of Cases of thyroid cancer $\frac{p}{100{,}000} \times 5.4$	PAF AF x No of cases.	PAF % $\frac{PAF}{No\ of\ cases} x100$
166-170	168	40.19	2.39	0.58	18	$9.7x10^{-4}$	$5.63x10^{-4}$	58
171-175	173	-	-	-	-	-	-	-
176-180	178	42.62	2.48	0.60	30	$1.6x10^{-3}$	$9.6x10^{-4}$	60
181-185	183	43.80	2.52	0.60	6	$3.24x10^{-4}$	$1.94x10^{-4}$	60
186-190	188	45.01	2.56	0.61	30	$1.6x10^{-3}$	$9.8x10^{-4}$	61
191-195	193	-	-	-	-	-	-	-
196-200	198	47.38	2.64	0.62	108	$5.8x10^{-3}$	$3.6x10^{-3}$	62
201-205	203	48.52	2.68	0.63	36	$1.9x10^{-3}$	$1.2x10^{-3}$	63
206-210	208	49.73	2.72	0.63	54	$2.9x10^{-3}$	$1.83x10^{-3}$	63
211-215	213	50.93	2.77	0.64	66	$3.6x10^{-3}$	$2.3x10^{-3}$	64
216-220	218	52.14	2.81	0.64	6	$3.24x10^{-4}$	$2.07x10^{-4}$	64
221-225	223	-	-	-	-	-	-	-
226-230	228	54.54	2.89	0.65	30	$1.6x10^{-3}$	$1.04x10^{-3}$	65
231-235	233	55.58	2.93	0.66	6	$3.24x10^{-3}$	$2.14x10^{-3}$	66
236-240	238	56.89	2.97	0.66	24	$1.3x10^{-3}$	$8.58x10^{-4}$	66
241-245	243	-	-	-	-	-	-	-
246-250	248	59.36	3.06	0.67	6	$3.24x10^{-4}$	$2.17x10^{-4}$	67
276-280	278	66.56	3.31	0.70	12	$6.48x10^{-4}$	$4.54x10^{-4}$	70
281-285	283	-	-	-	-	-	-	-
286-290	288	69.00	3.39	0.70	12	$6.48x10^{-4}$	$4.54x10^{-4}$	70
316-320	318	76.12	3.64	0.72	6	$3.24x10^{-4}$	$2.33x10^{-4}$	72
376-380	378	90.44	4.14	0.76	6	$3.24x10^{-4}$	$2.46x10^{-4}$	76
386-390	388	92.88	4.22	0.76	18	$9.7x10^{-4}$	$7.4x10^{-4}$	76
416-420	418	100.00	4.47	0.78	24	$1.3x10^{-3}$	$1.014x10^{-3}$	78
431-435	433	103.60	4.57	0.78	6	$3.24x10^{-4}$	$2.53x10^{-4}$	78
466-470	468	111.97	4.88	0.79	6	$3.24x10^{-4}$	$2.56x10^{-4}$	79
Total					**2,849,918**	**154**	**20**	**13**

Abbreviation:
CDI = chronic daily intake.
RR = relative risk.
AF = attributable fraction.
PAF = population attributable fraction.
Mg/kgbw/day = miligram per kilogram bodyweight per day.

The result of the risk estimates suggests that the relative risk (RR) of thyroid cancer increases as nitrate concentrations in drinking water increases and is greater than one (>1) in each exposure category. As the RR increases, the AF also increases and approaches its maximum value of one. The AF can range from a minimum value of zero to a maximum value of one (Rosen, 2013).

The result also suggest that of the 154 thyroid cancer cases calculated in a population of 2,849,918 in East Anglia, exposure to nitrates in drinking water (within the nitrate dose range encountered in drinking water in the region) is attributable to 20 cases or 13 per cent of the thyroid cancer cases and this would have been eliminated if there were no nitrate exposures in drinking water in the region. The 154 thyroid cancer cases are within the 320 cases reported for East of England (including East Anglia) for 2014 (ONS, 2016). Assuming 'low dose' as defined by USA National Toxicological Program (NTP, 2001) is the dose range below the LOAEL (i.e. 69mg/l as reported in the epidemiological studies), lifetime excess risk of thyroid cancer ranged from 0.02 to 0.38 (2 per cent to 38 per cent). Also, assuming 'low dose' is a dose range below the drinking water standard of 50mg/l, the excess lifetime risk is between 0.02 to 0.28 (2 to 28 per cent). The lifetime excess risk of thyroid cancer in the population in East Anglia within the dose range of nitrates (<2 to 95mg/l, excluding the outlier of 274mg/l) reported in the epidemiological studies (source populations) is 0.02 - 0.45 (2 - 45 per cent).

The increasing PAF% with increasing nitrate concentration indicates that exposure to nitrate doses at and below the drinking water standard of 50mg/l was attributable to 2 per cent to 28 per cent of the lifetime excess risk of thyroid cancer and this could have been eliminated if there was no exposure to nitrates in drinking water at this dose range. At nitrate doses greater than 50mg/l (>50mg/l), the lifetime excess was 30 per cent to 79 per cent and this could have been eliminated if there was no exposure to nitrates in drinking water at this dose range. As noted by Rockhill et

al, (1998), the PAF will increase with increasing exposure provided each exposure category has a RR >1. Although the excess lifetime risk of thyroid cancer by individuals in the study population is lower at nitrate levels below the drinking water standard, the number of thyroid cancer cases was higher when compared with the number of cases in the exposure categories above the drinking water standard where the excess risk was much higher. The reason for this may be because there were more people in the exposure categories below the drinking water standard of 50mg/l than in the exposure categories above the drinking water standard. While nitrate concentration in public water is much lower than in PWS, the population using public water is far higher than those on PWS and as a result, there were more people in the exposure category below the drinking water standard and therefore at a lower risk of thyroid cancer than those above the standard where the risk of thyroid cancer was much higher. The majority of the population in the lower exposure categories are public water users whilst the majority on the higher exposure categories are PWS users. But given that a population with many individuals at small risk cannot be distinguished from one with few individuals at high risk (Grau et al, 2010), the relative risk can be very important in any intervention and/or in designing public health policy (Walter, 1976; Wacholder et al, 1994). Whilst the RR increases with increasing nitrate concentration, a relative risk >1 in all the exposure categories suggest that exposure to nitrates in drinking water is associated with thyroid cancer and both PWS and public water users are at the risk of thyroid cancer although the risk is higher in the former than in the latter. This implies that the current policy in the UK as contained in the PWS Regulation (2009) where PWS serving single dwellings are exempt from the mandatory risk assessment which applies to all other category of PWS may put the health of the people living in such single dwellings at risk of thyroid disorders including thyroid cancer and therefore warrant a review.

Although the public health or societal impact of an exposure does not depend only on the magnitude of the relative risk, but also on the prevalence of the risk factor in the population (Macera & Powell, 2001), widespread contamination of drinking water sources by nitrates in the study population and the risk of thyroid cancer at exposure levels below and above the drinking water standard of 50mg/l suggest that everybody in the study population is exposed to nitrates in drinking water, albeit at different doses. The risk of thyroid disorders including thyroid cancer at nitrate doses below and above the drinking water standard supports the assumption of a non-threshold for nitrates and also supports the low-dose effect hypothesis. Prolonged exposure to nitrates in drinking water can result in iodine deficiency and decreased iodine uptake by the thyroid (Tonacchera et al, 2004). Even in the presence of sufficient iodine intake, prolonged exposure to nitrates in drinking water above the drinking water standard of 50mg/l can result in thyroid disorders including thyroid hypertrophy (goitre) and hyperplasia. However, even when nitrate concentration is below the drinking water standard, prolonged exposure can also result in thyroid disorders including goitre if there is insufficient iodine intake (van Maanen et al, 1994). Elimination of nitrates from the body is low following prolonged exposure to high concentrations of nitrates (van Maanen et al, 1994).

Appendix 9 shows the excess risk of thyroid cancer in the different age groups and suggest that the excess risk of thyroid cancer below and equal to the drinking water standard in infants is $9.9x10^{-3}$ to 0.14; children (1-6 years) is $9.9x10^{-3}$ to 0.11; children (7-12 years) is 0 to 0.06; adolescents (13 – 18 years) is 0 to 0.02; adults 0 to 0.03. Above the drinking water standard and within the nitrate dose range reported in East Anglia, the excess risk of thyroid cancer in the different age groups is 0.15 to 0.62 (infants); 0.11 to 0.53 (children 1- 6 years); 0.06 to 0.37 (children 7-12 years); 0.03-0.19 (adolescents); 0.03 to 0.23 (adults). Although the excess risk of thyroid cancer below and above the drinking water standard is higher in infants and children than

in adolescents and adults and consistent with the observation in the exposure assessment (Section 6.1.1) that nitrate body burden is higher in infants and children than in adolescents and adults, none of the children aged 0-5 years were served with tap-water from PWS in households where the source of drinking water is PWS (Table 4, Section 6.1.1).

7.4 DISCUSSION

Risk estimates suggest that of the 154 thyroid cancer cases calculated for East Anglia in 2014, the lifetime excess risk as a result of exposure to nitrates in drinking water was 20 cases or 13 per cent and this could have been eliminated if there were no nitrates in drinking water. At nitrate levels at or below the WHO/EU drinking water standards of 50mg/l, the lifetime excess risk of thyroid cancer is 0.02 to 0.28. Health Canada (2013) estimated lifetime excess risk of cancer from endogenous nitrosation of nitrosodimethylamine (NDMA) in the stomach after exposure to nitrates in drinking water at the USA/Canada drinking water standard of 45mg/l and reported excess risk of $1.6x10^{-6}$. This is above the range of $1x10^{-6}$ to $1x10^{-5}$ considered to be negligible by Health Canada (Health Canada, 2013). Although the lifetime excess risk of 0.02 to 0.28 (2- 28 per cent) for thyroid cancer at the WHO/EU drinking water standard of 50mg/l is higher than in the Canadian study, it must be noted that whilst the Canadian study was based on the mechanism of endogenous nitrosation between nitrates and amines and or amides (nitrosatable precursors) in the stomach resulting in the formation of carcinogenic N-nitroso - compounds, this study is based on the thyroid gland iodine uptake inhibition mechanism.

The finding that the excess risk of thyroid cancer is higher in infants and children than adolescents and adults is consistent with the finding in the exposure assessment (Section 6.1.1) that nitrate body burden is more on infants and children than adults. The high nitrate body

burden in infants and children is also consistent with the view that infants are more sensitive to the effect of nitrates in drinking water than adults (Gatseva & Argirova, 2005; 2008a&b). Infants and children are more sensitive to the effects of iodine inhibitors than adults (Tajtakova et al, 2006; Radikova et al, 2008; Health Canada, 2013) and therefore require more iodine intake in order to produce more thyroid hormones during shortages (Health Canada, 2013). Although the excess risk of thyroid cancer at nitrate levels below and above the drinking water standard is higher in infants and children than in adolescents and adults, none of the children aged 0-5 years in the study area were served with tap-water from PWS in households where the source of drinking water is usually PWS (Table 4). This may be due to the Advice Notes (Appendix 8) issued by the Council's Environmental Health Officers; the Health Protection Agency (now Public Health England) and Midwives to expectant mothers and mothers with younger children in households served by PWS on the association between nitrates in drinking water and infantile methaemoglobinemia; and the need to use alternative sources of drinking water especially in preparing infant formula if nitrates are found to be above 50mg/l in PWS. While children in households served by PWS used tap-water from the age of 6years (no other source of water was indicated in the questionnaire), children in households served by public water used tap-water from <1year. The ONS in its cancer records for 2014 for England (the latest thyroid cancer records) reported no thyroid cancer cases in children less than one year (<1 year). Only three thyroid cancer cases were recorded in children 1- 9 years and these cases were in females only (ONS, 2016). Cases of thyroid cancer increased from age 10-14years and above according to ONS (2016).

In the UK, thyroid cancer incidence rate has increased by 149 per cent since the late 1970s. The increase is larger in females (164 per cent) than in males (152 per cent) (Cancer Research UK, 2016). Over the last decade, thyroid cancer incidence rate have increased by more than two-

thirds (71 per cent) in the UK, and includes similar increases in females (73 per cent) and males (70 per cent). Between 2014 & 2035 thyroid cancer is projected to rise by 74 per cent in the UK i.e. 11 cases per 100,000. It is projected that 1 in 480 men and 1 in 180 women may be diagnosed with thyroid cancer during their lifetime in the UK (Cancer Research UK, 2016). In England, the number of thyroid cancer cases in 2014 was 2,941 (male =826; female = 2115) (Cancer Research UK, 2016). A breakdown of these cases in regions (Figure 10) shows that East of England, (including East Anglia) was among the regions with higher incidence rates (320) in 2014. The highest incidences rate was reported in the Northwest (442); London (483) and the Southeast (348). The incidence in Yorkshire & Humber was 300; West Midlands (298), East Midlands (219); Southwest (256) while the least, 129 was recorded in the Northeast (ONS, 2014).

The increasing thyroid cancer incidence in the UK is also reported in the rest of the European Union. According to Cancer research UK (2014), about 33,600 new cases of thyroid cancer were diagnosed in the 27 countries of the Union (EU-27) in 2008 with the highest incidence rate (18.6 per 100,000) reported among females in France. The lowest incidence rate was in Greece, 3.3 per 100,000. The age- standardisation rate in the UK was however lower than the EU average (Cancer Research UK, 2014). Around the world, thyroid cancer cases occur more in females aged 15-44 than in any other age group (Ferlay et al, 2008). The highest incidence rate has been reported in North America among females in whiles the lowest incidence was recorded in Africa, with about 1.2 cases per 100,000 females reported in middle Africa (Ferlay et al, 2008).

While the reasons for the large increases in thyroid cancer especially in females are unclear, Davies & Welch (2006); Olaleve et al (2011) have suggested that the increased incidence tend to reflect better detection of subclinical disease rather than a true increase in incidence. However,

Chen et al (2009) and Aschebrook-Kilfoy et al (2011) were of the opinion that improved and increased diagnostic activity cannot completely explain the increase in rates. While the only established risk factor for thyroid cancer is exposure to iodising radiation especially in childhood (Ron et al, 1995), pre-existing thyroid disorders including goitre or benign thyroid adenoma (Franceschi et al, 1999), family history, especially in first degree relatives (Nose , 2010) have been reported as risk factors of thyroid cancer. According to Zhao et al (2012); Bloomberg et al (2012) and Enewold et al (2009), risk factors such as use of medical diagnostic radiation; obesity; changes in the pattern of iodine fortification of salt; and other unknown risk factors may be contributing to the increased thyroid cancer rate. Also, genetic and environmental factors have also been implicated in the aetiology of thyroid cancer (Balasurbramaniam et al, 2012).

However, the findings that the lifetime excess risk of thyroid cancer (0.02 to 0.28) at nitrate levels below and equal to the drinking water standards of 50mg/l is above the range ($1x10^{-6}$ to $1x10^{-5}$) considered negligible by Health Canada suggests that exposure to nitrates in drinking water is a risk factor for thyroid cancer and highlights the public health implications of exposure to nitrates in drinking water. Some environmental factors that have been linked with thyroid cancer included diet, tobacco use, alcohol intake (Ron & Schneider, 2006) and nitrate exposures (Pellegritti et al, 2013). As reported by Peterson et al (2012), apart from iodine deficiency, dietary factors that interfere with iodine uptake and production of thyroid hormones can have effect on thyroid cancer risks. Similarly, certain environmental pollutants such as nitrate exposures that can compete with iodine uptake by the thyroid gland are potential thyroid function disruptors and carcinogens (Pellegritti et al, 2013). Although a number of environmental contaminants can interfere with thyroid hormone function resulting in goitre, benign nodules and cancer, some of these pollutants which occur in water, air or food could act competitively or in synergy to induce thyroid disease (Leux & Guenel, 2010). As noted by Mukhopadhyay et al (2005) and Gatseva & Argirova (2008b), the persistence of residual goitre

in some countries despite the successful implementation of salt iodization programmes suggests that nitrates in drinking water may be interfering with iodine uptake by the thyroid gland. In a cumulative risk assessment of the three most important iodine inhibitors found in drinking water (thiocyanate, perchlorate and nitrate) using the perchlorate equivalent concentration (PEC) ratio of 1: 15: 240 or 1: 8: 150 for $Cl0_4^-$: SCN^- : NO_3^- , De Groef et al, (2006) reported that exposure to nitrates at the drinking water standard of 50mg/l, induced iodine inhibition 12times greater than perchlorate at its recommended standard of 0.007mg/kg/day (equivalent to 24.5ppb). Similarly, in Belgium, De Groef et al, (2006) also reported that exposure to 12.3mg/lNO_3^- (equivalent to 82ppb perchlorate) and <2µg/l CN^- (equivalent to <0.23ppb perchlorate) will induce iodine inhibition 3-times greater than that of perchlorate at the recommended RfD. Therefore, if an adult consumed about 2L of such water per day (assuming it contained perchlorate at the RfD of 24.5ppb), nitrates would be contributing about 77 per cent of the total iodine inhibition while 23 per cent would be contributed by perchlorate (De Groef et al, 2006). These findings suggest that lowering the level of nitrate exposure in drinking water is a more effective approach to lowering exposure to iodine inhibition load.

Although higher nitrate levels have been found in PWS than in public supply, a comparison of thyroid cancer incidence in England in 2014 and the number of PWS in the regions Table 10 indicate that regions with high levels of thyroid cancer cases such as London/Southeast; Northwest; East (including East Anglia); Yorkshire & Humber and the West Midland also have high number of PWS, according to the PWS records for 2013 (DWI 2013). Although the Southwest has the highest number of PWS, the number of thyroid cancer cases was lower than in the Northwest or East (including East Anglia). Given that nitrate levels in PWS are usually higher than in public water, it is not known if there are clusters of thyroid disorders including

thyroid cancers cancer in regions of the UK with high number of PWS and this should be investigated.

Table 10: Number of PWS (2013) and thyroid cancer cases by Region in England (2014)

REGION	**No of PWS (2013)**	**Thyroid cancer cases (2014)**
North East	1891	129
North West	6144	442
Yorkshire/Humber	5051	300
East Midlands	1581	219
West Midlands	6595	298
East of England	5285	320
London/South East	2690	977
South West	15,309	256
Total	**44,546**	**2941**

7.4.1 PUBLIC HEALTH IMPLICATIONS

The public health implications of exposure to nitrates in drinking water stems from the ability of nitrates to inhibit iodine supply to the thyroid gland by competitively binding to the sodium (Na^+)/iodide (I^-) symporter (NIS) on the surface of the thyroid follicle, thus resulting in iodine

deficiency (Jahreis et al, 1986; Dohan et al, 2003; Tonacchera et al, 2004; Braverman et al, 2005; Ward et al, 2010; Wilson, 2010). The NIS is a protein membrane that mediates the transport of iodine from the blood into the thyroid (Wilson, 2010). Whilst iodine, which is obtained primarily from diet (Brantsaeter et al, 2013), plays a crucial role in the synthesis of thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) and constitutes about 65 per cent and 59 per cent of their respective weight (Andersson et al, 2007a); prolonged exposure to nitrates in drinking water can lower the total iodine uptake (TIU) by the thyroid gland resulting in decreased production of thyroxine (T_4) and triiodothyronine (T_3) (Tonacchera et al, 2004; Wilson, 2010). Thyroid hormones regulate metabolic processes in most cells as well as play a role in the growth and development of most organs especially the brain early in life. Therefore, iodine inhibition during foetal development and in the first two to three years of life that results in decreased synthesis of thyroid hormones can lead to hypothyroidism and brain damage (Andersson et al, 2007a); the clinical consequence of which will be irreversible mental retardation (Delange, 2001).

The ability of nitrates to inhibit iodine uptake by the thyroid gland appears to be influenced by the amount of dietary iodine intake. Prior to 1930s, iodine deficiency and goitre were endemic in parts of the UK (Phillips, 1997). However, the addition of iodine to cattle feed to increase milk production which incidentally led to increased iodine concentration in milk and dairy products and the policy of successive governments since the 1940s of encouraging milk consumption especially among school-age children led to the eradication of iodine deficiency disorders (IDD) in what was described as "an unplanned and accidental public health triumph" (Phillips 1997: p.391). However, recent studies suggest that iodine deficiency has re-emerged in the UK, especially among schoolgirls (Vanderpump et al, 2011; Vanderpump, 2012; Andersson et al, 2012; UNICEF, 2012; Pearce et al, 2013); women of child-bearing age and pregnant women (Kibridge et al, 2004; Bath et al, 2008; Pearce et al, 2010; Pearce et al, 2013; Bath et al,

2013). The UK was seventh among the top ten iodine deficient countries in 2011 based on a national median urinary iodine of <100µg/l among school-age children (Andersson et al, 2012). While the reason for the re-emergence of iodine deficiency is unclear, Vanderpump et al (2011) and Vanderpump (2012) suggest that the re-emergence of iodine deficiency may be due to the decline in milk consumption by the population. Milk contributes about 41per cent of the total iodine intake in the UK (Food Standards Agency, 2008) and the concentration of iodine in milk in the UK has remained unchanged (Vanderpump et al, 2011). Although the decline in milk consumption may well be a factor in iodine deficiency in the UK, the findings from this study that nitrates in drinking water is a risk factor for goitre and risk of thyroid cancer through the mechanism of iodine uptake inhibition by the thyroid gland cannot be ignored given widespread nitrate contamination of drinking water sources in the UK and in many countries of the world. This raises some public health concerns especially for pregnant women and children who are more sensitive to nitrates exposures. According to Wilson (2010), the thyroid is less able to tolerate high exposure to iodine inhibitors (e.g. nitrates) in the presence of decreased dietary iodine intake. In other words, the body's ability to cope with exposure to iodine inhibitors decreases rapidly as iodine intake decreases. Adequate iodine intake is therefore critical in the public health implications of nitrate exposure (Wilson, 2010). However, given that "dietary iodine intake is entirely opportunistic in the absence of iodine supplementation" (Smyth et al, 2013: pg1), iodine supplementation in the form of salt iodization (addition of iodine to salt) under the universal salt iodisation (USI) programme have been adopted to eradicate endemic iodine deficiency and goitre by countries such as the United States (Pearce et al, 2004); Switzerland (Andersson et al, 2010); Australia and New Zealand (Mackerras & Eastman, 2012; DePaoli et al, 2012), Denmark (Rasmussen et al, 2008), Belgium (Vandevijvere et al, 2012), Switzerland (Andersson et al, 2010) and Norway (Brantsaeter et al, 2013). Also in countries such as the USA and Switzerland which are considered to be iodine sufficient, household iodised salt,

iodised bread and milk are the major dietary sources of iodine (Caldwell et al, 2011; Hadilman et al, 2005). No salt iodisation programme has ever been undertaken in the UK (Philip, 1997; Vanderpump et al, 2011; Bath et al, 2013) as the iodine content of milk alone was judged to be almost sufficient to meet the recommended daily iodine requirement of 150µg/day (Phillips, 1997; Vanderpump, 2012). Household iodised salt is rarely available to purchase and few manufacturers, if any, use iodised salt in the manufacture of food .e.g. bread (Lazarus & Smyth, 2008). However, the decline in milk consumption in the UK (European Commission cited by Vanderpump et al, 2011) and the evidence from this study that exposure to nitrates in drinking water is a risk factor for goitre and thyroid cancer are of significant public health importance. The findings provides information that can be used by policy makers to review the current policy on iodine prophylaxis e.g. salt iodisation and iodine supplementation in the UK, and supports the call by Vanderpump et al (2011: pg. 2011), "for a comprehensive survey of the UK iodine status and the urgent need for the implementation of iodine prophylaxis". Given that no national survey has been undertaken since the 1940s to monitor the iodine status of the UK population (Vanderpump et al, 2011; Bath et al, 2013); this review will help to put iodine back on the public health agenda. As noted by Bath et al (2013: pg. 336), "iodine has not been part of the UK public health agenda for the past 50 years; guidelines on iodine requirement during pregnancy and lactation are out dated; advice to pregnant women has not included information about iodine intake and monitoring of iodine status in the UK population has been absent". According to Vanderpump (2012: pg.3), "if we wish government to consider iodine prophylaxis to offset the adverse effect of iodine deficiency, data is required to provide reassurance that at the population level, the benefits far outweigh any disadvantages".

While improving nutritional iodine intake is an important public health issue, it is an incomplete response to the adverse effects of exposure to nitrate contaminated drinking water. The evidence

that nitrate is a risk factor of thyroid disorders, especially goitre and the findings of thyroid cancer cases at nitrate levels below and equal to the drinking water standard of 50mg/lNO_3, suggests that the current drinking water standard of 50mg/lNO_3, originally set to protect against infantile methaemoglobinemia, is unlikely to protect against thyroid disorders including goitre and warrants a review. The review should include a consideration of setting the drinking water standard as low as reasonably practicable (ALARP) in order to decrease exposure to this ubiquitous iodine inhibitor and to protect the health of the public, especially pregnant women and infants from iodine inhibition and its consequences. Also, it will help to protect the health of the population whose source of drinking water is from PWS where nitrate concentrations are higher compared with public water supplies. The ALARP principle has been adopted for non-threshold substances to diminish minimal risks (DEFRA & Environment Agency, 2002). Although nitrate is a weak iodine inhibitor compared to perchlorate and thiocyanate (Braverman et al, 2005; Wilson, 2010), human exposure to nitrate is far greater than perchlorate and thiocyanate, and accounts for the majority of the body's total iodine inhibition load in adults, foetuses and infants (Wilson 2010). Therefore, "lowering the public's nitrate exposure provides a more meaningful opportunity to lowering the public's iodine inhibition load and risk" (Wilson 2010: pg182).

7.5 UNCERTAINTIES AND LIMITATIONS OF THE STUDY

This study is limited by assumptions and uncertainties and as a result may not have predicted the excess risk of thyroid cancer with certainty. It is important therefore that uncertainties associated with the risk estimates are characterised, understood and recognised in any policy decision. Risk assessment and the science underlying its assumptions have uncertainties and are therefore not able to predict risk with certainty (Tukker, 2002; Gochfeld, 2003; USEPA, 2004). Uncertainty is

defined as lack of precise knowledge of what the truth is (USEPA, 2004; Bois et al, 2006) and a discussion of uncertainty inherent in the risk assessment process is necessary for a better understanding of the implications and limitations of the study findings (USEPA, 1992). Characterisation or assessment of uncertainty aims to identify the sources of uncertainty, understand and quantify them and devise ways of reducing it (Bios et al, 2006, Van Asselt et al, 2001). The results of such assessment can contribute to a discussion on the quality of information underpinning a policy decision, determine the extent of intervention necessary and help allocate resources for monitoring and or further research (USEPA, 2004; Von Krauss & Martuzzi, 2006) and this therefore a very essential step in decision-making (USEPA, 2004; Bois et al, 2001). According to Walker et al (2003), uncertainty can be considered as a two dimensional concept, consisting of location and level. Whilst the location of uncertainty refers to where uncertainty manifest in the risk assessment process, the level of uncertainty refers to the degree of severity of uncertainty characterising the risk assessment process as seen by the decision-maker (Van der Sluijs et al, 2003; Von Krauss & Martuzzi, 2006).

Uncertainty in risk assessment can be assessed qualitatively; quantitatively or both (USEPA 1992, 2004, IRR 2005b, Van Asselt et al 2001, Van der Sluijs et al 2003). Whilst the qualitative approach involves characterising the location and sources of uncertainty as well as estimating the level or severity, the quantitative approach aims to evaluate the extent to which a particular uncertainty impacts on the risk estimates (Van Asselt et al, 2001; USEPA, 1992 & 2004). In this study, uncertainty associated with the risk estimates was only assessed qualitatively as presented in Table 11 below. The assessment was carried out following the Walker & Harremoes (W&H) framework as described by Van der Sluijs et al (2003); Janseen et al (2005) and Von Krauss & Martuzzi (2006). This framework has been adopted by the Netherlands Environmental Assessment Agency (MNP/RIVM, Guidance for Uncertainty Assessment and Communication)

(Van der Sluijs et al, 2003; Janssen et al, 2005) and has been applied in the risk assessment of Genetically Modified Crops (Von Krauss 2005) and the health impact assessment of particulate matter (Petersen et al, 2006). It was also proposed in the Integrated Assessment of Health Risks of Environmental Stressors in Europe (INTARESE) (Von Krauss and Martuzi 2006). Van Grinsven et al, (2010) have conducted a qualitative uncertainty assessment of colon cancer risk as result of exposure to nitrates in drinking water.

The assessment (Table 11) shows that there are uncertainties in the hazard identification, dose - response, exposure assessment stages of the risk assessment which may have affected the risk estimates. Uncertainty in risk characterisation is as a result of uncertainties in the preceding stages of the risk assessment. Also, there are uncertainties in the risk estimates as a result of variability. Variability refers to inter-and-intra individual differences inherent in a study population (USEPA, 2004). These differences include age, gender, height, genetic variations, social economic status, body weight etc. It also includes differences in exposure and sensitivity evident in a population.

Table 11 OVERVIEW OF UNCERTAINTY

SOURCES/LOCATION OF UNCERTAINTY	EVALUATION
Hazard Identification	Inconsistency in the epidemiological studies used in deriving the risk estimates. The inconsistency may be due to: • Differences in the study design • Differences in the concentration of nitrates in drinking water. • Differences in the duration of exposure to nitrates. • Differences in exposure to other risk factors. • Individual variability. • Assumption of causality between nitrates and goitre.
Dose-Response Relationship	• Assumption of low dose linearity (model uncertainty). • Assumption of linearity at high dose • Assumption of non-threshold effect between nitrates and goitre • Different definitions of low dose.
Exposure Assessment	Nitrate concentration data used in the exposure assessment as obtained from water companies and Local Authorities may be a source of error in the risk estimates. This may be due to: • Sampling, measurement or recording error by water companies. • Sampling or water sample collection (PWS) error by Local Authority Environmental Health Officers. • Error in laboratory analysis of water samples by water companies or Local Authorities • Misclassification of exposure (incorrectly assigning PWS users as public supply users or vice versa). • Seasonal variations in nitrate levels in drinking water sources.

Table 11 (Continued): OVERVIEW OF UNCERTAINTY

SOURCES/LOCATION OF UNCERTAINTY	EVALUATION
Variability	• Differences in life style, genetic make- up, body weight, age, sex, etc. • Differences in individual exposure to nitrates (amount of tap-water intake). • Differences in individual sensitivity to nitrate exposure.
Risk Characterisation	• Uncertainty in risk characterisation is a sum of uncertainties in the hazard identification, does-response & exposure assessment. • Use of thyroid cancer as a proxy for goitre was unavoidable given that there is no register for goitre cases in the UK.

While the level of uncertainty ranges from what is 'known with certainty to what is unknown', assessing uncertainty qualitatively provides an overview of where the most policy relevant uncertainty is located and can help to determine where elaborate uncertainty assessment is required (Petersen et al, 2006). The assessment suggests that the most policy relevant uncertainties are located at the hazard identification and exposure assessment stages. While uncertainties as a result of inconsistent epidemiological studies; water sampling or nitrate measurement error by water companies or local authorities; error in laboratory analysis of water samples or exposure misclassification can further be analysed quantitatively to determine the impact of each of these uncertain factors on the risk estimates, well designed epidemiological studies can help to reduce the levels of inconsistency in future studies on the health effect of nitrates in drinking water. Improved nitrate data collation and laboratory analysis of water samples can help to reduce uncertainty at the exposure assessment stage. Model uncertainty and seasonal variations on the other hand cannot be quantified or reduced, but need to be recognised

and taken into consideration in any policy decision involving these risk estimates. Also, variability which is an attribute of nature cannot be reduced by collection of additional data or analysis (USEPA, 2004) but need to be taken into consideration in any policy decision.

Model uncertainties are due to the inability of the linear non-threshold model to correctly describe the mechanism or mode of action of nitrates on the thyroid (especially at low dose) leading to thyroid disorders, including thyroid cancer. The inability of mathematical models which are sometimes used in risk assessment in the form of equations to correctly describe a real situation is as a result of limited knowledge of the toxicokinetics and/ or toxicodynamics of an agent leading to a health effect (Finkel, 1990; Morgan & Henrion, 1990; USEPA, 2004; Von Krauss & Martuzzi, 2006). Limited knowledge is not the absence of knowledge but imperfect knowledge of the causal relationship underlying a health outcome (Bois et al, 2006), and is usually due to the state of our knowledge rather than the fault of the risk assessor (Van Asselt et al, 2001).

Despite the uncertainties associated with the risk estimates as outlined above, this study is strengthened by the use of actual nitrate concentrations in public supplies and PWS from the study area in the exposure assessment. Also, the use of data on the amount of tap-water intake obtained directly from individuals in the study area (including those on PWS) in the exposure assessment further strengthens the findings of the study.

7.6 CONCLUSION

Although the weight of evidence assessment suggests that exposure to nitrates in drinking water is strongly associated with goitre, thyroid cancer was used as a proxy for goitre in the risk

estimates because there is no register of benign thyroid tumour in the UK. There is a close link between benign and malignant tumours as they share common aetiological factors. Also, the existence of a goitre or nodule of the thyroid is associated with excess risk of thyroid cancer. Cases of malignancy can develop from goitre which is usually benign.

Risk estimates suggest that 20 cases or 13 per cent of the 154 thyroid cancer cases calculated for East Anglia in 2014 could be attributable to exposure to nitrates in drinking water and this could have been eliminated if there were no nitrates in drinking water. At nitrate levels below and equal to the WHO/EU drinking water standards of 50mg/l, the lifetime excess risk of thyroid cancer is 0.02 to 0.28. This is above the range considered as negligible by Health Canada. The risk of thyroid cancer at nitrate levels below and above the drinking water standard suggests that there is no threshold for nitrate exposures on the thyroid gland and supports the low-dose effect hypothesis. Although the excess risk of thyroid cancer is higher amongst PWS users than public water users, the number of public water users is far higher than the number of PWS users. East Anglia is one of the regions in the UK with high number of PWS as well as high incidence rate of thyroid cancer according to 2014 cancer records but it is not known if there are thyroid cancer clusters in this region or other regions of the UK with high number of PWS and high numbers of thyroid cancer cases. This warrants further investigation. The implications of the risk of thyroid cancer and nitrate levels below and equal to the drinking water standard is that the current drinking water standard of 50mg/l, originally set to protect against infantile methaemoglobinemia (blue - baby syndrome), is unlikely to protect against thyroid cancer and warrants a review. Although nitrate is a weak iodine inhibitor when compared to other iodine inhibition anions such as perchlorate or thiocyanate, human exposure to nitrates is far greater than perchlorate or thiocyanate and accounts for the majority of the body's total iodine inhibition load in adults, foetuses and infants. Therefore the review of the current drinking water standard

should include a consideration of lowering the standard in order to decrease exposure to this ubiquitous drinking water contaminant and protect public health, especially for infants, children and pregnant women who are more sensitive to nitrate exposure. Also, it will help to protect the health of the population whose source of drinking water is from private water supplies where nitrate concentrations are higher, than in public water. Lowering nitrate exposure in drinking water is a more effective approach to lowering the body's iodine inhibition load.

The public health implications of exposure to nitrates in drinking water stems from the ability of nitrates to interfere with iodine uptake by the thyroid gland resulting in iodine deficiency; low thyroid hormone production; goitre and in some cases, thyroid cancer. Thyroid hormones play an important role in growth and development early in life and iodine inhibition that occurs during foetal development and in the first three years of life can result in irreversible brain damage.

The UK is one of the top ten iodine deficient countries in the world. Iodine deficiency in the UK is classed as moderate -to -mild and is based on median urinary iodine concentration of <100µg/l among school-age children. Although decline in milk consumption has been suggested as a possible reason for the deficiency, exposure to nitrates in drinking water may be a risk factor given the capability of nitrates to disrupt iodine uptake by the thyroid gland and widespread nitrate contamination of drinking water, especially PWS.

The ability of nitrates to inhibit iodine uptake by the thyroid gland resulting in thyroid disorders, including goitre and possibly thyroid cancer appears to be influenced by the amount of dietary iodine intake. Given that chronic exposure to low - dose of nitrates (<50mg/l) can result in thyroid disorders if there is severe iodine deficiency, chronic exposure to elevated levels of nitrate can also result in thyroid disorders (goitre) even when iodine intake is sufficient. This may be due to the poor elimination of nitrates from the body after prolonged exposure to

elevated levels of nitrates. This may explain the persistence of goitre in some countries that have successfully implemented iodine prophylaxis in the population e.g. salt iodization programmes. Given that the effect of nitrates on the thyroid gland is moderated by iodine, adequate dietary iodine intake is therefore critical in public health protection from the adverse effects of nitrates since the ability of the body to cope with iodine inhibitors decreases as iodine intake decreases. But given that reliance on dietary iodine intake alone cannot guarantee adequate supply of iodine to the body, iodine prophylaxis in the form of salt iodisation, iodised bread, iodised oil etc. has been adopted by many countries to eradicate endemic iodine deficiency and goitre. No iodine prophylaxis programme, for example, salt iodisation programme has ever been carried out in the UK, but in the face of declining milk consumption by the population and widespread nitrate contamination of drinking water sources, iodine prophylaxis in the form of salt iodisation or other iodine supplements can be used to address the adverse effect of nitrate exposure in the UK.

CHAPTER EIGHT

8.0. DISCUSSION, CONCLUSION AND RECOMMENDATIONS FOR FURTHER STUDIES

The main aim of this study was to determine the nature of any thyroid disorders resulting from exposure to nitrates in drinking water with reference to public and private water supplies and to quantify the risk in East Anglia. This chapter discusses the relationship between the study findings and previous work detailed in the literature review; the study objectives and any new research appearing since the study began. It also addresses the implications of the research findings on prevailing public health policy as it relates to exposure to nitrates in drinking water and recommends areas for further studies. The IARC in 2010 classified ingested nitrate and nitrite as a probable human carcinogen (Group 2A cancer classification) on the basis of insufficient human evidence but sufficient evidence from experimental animal studies. This classification formed the basis of this study given widespread nitrate contamination of drinking water sources in some regions of the UK including East Anglia. The study followed the risk assessment framework. Quantitative risk assessment provides evidence - based risk estimates (evidence –based public health) for thyroid disorders due to nitrates exposures in drinking water which can inform policy decision making.

Access to drinking water that is wholesome (clean, adequate, safe) is essential for health, a basic human rights and a major component of public health policy. The study objectives therefore included a review of published epidemiological and experimental animal studies and case-reports for evidence of thyroid disorders as a result of exposure to nitrates in drinking water; to describe any evidence of association; to determine if any association is causal and to describe the

mechanism of action of nitrates on the thyroid gland. Also, it aimed to determine the concentration of nitrates in drinking water in the study area (East Anglia, UK); the frequency and magnitude of exposure as well as the amount of tap-water intake by the population. It concludes by evaluating the appropriateness of the current drinking water standard of 50mg/l (originally set to protect against infantile methaemoglobinemia) in protecting against thyroid disorders. What follows are the most significant findings from the study and the most probable conclusions in my opinion.

8.1 SUMMARY OF THE FINDINGS

1. HAZARD IDENTIFICATION

There is widespread contamination of drinking water sources especially PWS in some regions of the UK including East Anglia. A review of animal and epidemiological studies suggests that exposure to nitrates in drinking water is associated with moderate - to - mild iodine deficiency; hypothyroidism; hyperthyroidism; goitre and thyroid cancer. However, following a meta - analysis, the weight of evidence is strongest for goitre (effect estimate, OR = 3.13); weak for subclinical hypothyroidism (OR = 1.23) and weakest for clinical hypothyroidism and hyperthyroidism (clinical and subclinical). The effect estimates shows that the risk of goitre is more than 3 times higher in the exposed group than control group and suggest that exposure to nitrates in drinking water is a risk factor for goitre. The goitrogenic effect is mostly related to nitrate levels equal to or greater than 50mg/l (≥50mg/l) in the event of moderate - to - mild iodine deficiency. In a review, WHO (2011), reported that the anti-thyroid effect of nitrates is mostly related to exposure in drinking water rather than diet. The effect is more profound if there

is iodine deficiency but weak if there is adequate nutritional iodine intake i.e. iodine intake determined by urinary iodine excretion of 150-300µg/day (WHO, 2011).

While the result of meta-analyses on clinical hyperthyroidism and clinical hypothyroidism are consistent with that of Bahadoran et al (2015), they were unable to conduct a meta- analysis on goitre citing lack of data. This may be due to the cross – sectional nature of the studies on goitre. Although there was insufficient data for a meta-analyses on moderate - to - mild iodine deficiency and thyroid cancer, the evidence is consistent with the mechanism of action of nitrates which stems from the ability of nitrate ions to inhibit iodine uptake by the thyroid gland by binding to the sodium – iodide symporter (NIS) on the surface of the thyroid follicles resulting in iodine deficiency, low thyroid hormone production and high TSH production. Chronic stimulation of the thyroid gland by TSH to produce more hormones in the event of low thyroid hormones could result in thyroid hypertrophy (goitre), hyperplasia, adenomas and carcinoma (Hiasa et al, 1991; Capen 1992; 1997). Although the results of the only two studies thyroid cancer are contradictory, there was insufficient data for a meta- analysis on this outcome. However, Bahadoran et al (2015) conducted a meta- analysis on thyroid cancer following exposure to nitrates from diet (including drinking) and reported a non-statistically significant association, RR= 1.36, (95%Cl: 0.67-2.75). The potential for thyroid cancer as a result of exposure to nitrates in drinking water is biologically plausible given that there is a close link between benign and malignant tumour as the two shares a common aetiological factor (Leux & Guenel, 2010). Although the mechanism of iodine inhibition in the aetiology of thyroid tumour is established in animals (Kanno et al, 1990; Hiasa et al, 1991; Capen, 1992; 1997), the same cannot be said for humans (Schnieder & Brenner, 2003; Croften, 2008). However, hypothyroidism is reported to be associated with increased risk of thyroid cancer in humans (Balasubramaniam et al, 2012). While TSH is a known risk factor for thyroid nodules, continued

TSH stimulation of the thyroid to produce more hormones in the event of thyroid hormone shortages is reported to be associated with initiation and/- or promotion of thyroid carcinoma in humans (Johklass et al, 2008). Endogenous nitrosation which can result in the production of carcinogenic NOCs may be a mechanism for nitrate carcinogenicity in humans (IARC, 2010). NOCs have been reported to induce cancer at various organ sites (including the thyroid) in animals (Bogovski & Bogovski, 1981) and in humans (ASTDR, 2001; Weyer, 2003).

Although a cause - and- effect relationship has not been firmly established between nitrates in drinking water and goitre, the risk assessment framework can be used to estimate the lifetime excess risk of this outcome in East Anglian given widespread nitrate contamination of drinking water sources in the region.

2. DOSE - RESPONSE ASSESSMENT

A linear model is assumed for the relationship between nitrates in drinking water and goitre given that the effect of nitrates on the thyroid gland increases as dose increases and is judged to be best suited to describing the effect of nitrates on the thyroid gland at low dose. Low – dose extrapolation is important as it allows for risk estimation in the entire dose range of exposure of a substance in a population (Shepard et al, 1987). The ability of nitrates to disrupt iodine uptake by the thyroid gland resulting in low thyroid hormone production and thyroid hypertrophy (goitre) (Bloomfield et al, 196; 1962; Alexander & Wolff, 1966) suggests that nitrate is an endocrine disruptor. Given that the effect of nitrates is mediated by its metabolite, NO_x and not nitrite as previously thought (Addiscott & Benjamin, 2004), and the fact that NO_x is capable of exerting endocrine activity (Bryan et al, 2007; Elrod et al, 2008; Ghasemi & Zahedias, 2011) further supports the endocrine disrupting potentials of nitrates. There is no threshold for EDCs

(Sheehan & vom Saal, 1997; Crews et al, 2000). According to the USEPA, linearity at low- dose is to be assumed for substances whose toxicity is mediated by binding to a receptor (USEPA, 2000).

Given that nitrate is produced endogenously for some biological activities (Jaffe, 1981; Walker 1995; Hsia, 1998) such as antimicrobial activity (Hibbs et al, 1987; Fang, 1997; Addiscott & Benjamin, 2004), vasodilation (Bjorne, 2005), neurotransmission (Garthwaite, 1991), or immune regulation (Hibbs, 1991), any threshold would already have been exceeded for these effects to occur. Therefore exogenous nitrate exposure via drinking water which is reported to increase the concentration of endogenously produced nitrate as well as its toxicity (Berger et al 1997, Gupta et al 1998) suggests that there may be no threshold for nitrates especially on the thyroid gland. There is no threshold for EDCs (Sheehan & vom Saal, 1997; Crews et al, 2000) and nitrates as an endocrine disruptor may not be different. As noted by Welshons et al (2003), exogenous chemicals (e.g. EDCs) can influence a process that is already ongoing and thus is already above threshold, therefore there may be no threshold for toxic chemicals like EDCs. The linear model is recommended for low – dose extrapolation of non- threshold substances (Paustenbach, 1989; USEPA, 1989). It has been suggested that some substances, including EDCs, whose mechanism of action or toxicity is mediated by binding to a receptor can exhibit a non-monotonic dose-response relationship, (NMDR) where response (effect) increases and decreases as dose increases (Welshons et al (2003);Vandenberg et al, 2012). It is not known if nitrates can exhibit a NMDR and this warrants further investigation.

3. EXPOSURE ASSESSMENT

Higher nitrate concentrations have been found in PWS than in public water in the UK but the number of people exposed to PWS is far less than those exposed to public water. Although the Southwest England has the highest number of PWS in England, East Anglia has the highest number of people exposed to PWS. On average, the amount of tap-water intake in the study area was 0.95 L/day for infants (≤ 1 year); 1.05 L/day for children (1-6years); 1.0 L/day for children (7-12years); 0.8 L/day for adolescents (13-18years) and 1.2 L/day for adults (19-70years). Although there is no statistically significant difference in tap - water intake between PWS and public water users in the age groups, the average range of tap - water intake in this study area (0.8 - 1.2L/day) compares with the amount of tap-water intake reported in the rest of the UK population where an average consumer has been reported to consume about 1.14 L tap-water per day (DWI 1996); 0.70 - 1.9 L/day (Hunter et al, 2004); 0.9-1.3 L/day (Hopkin & Ellis; 1980); 0.10 L - 1.55 L/day (Mons et al, 2007), and also compares with the amount of 0.77 L - 1.44 L/day reported by Kaur et al (2004) among pregnant women in the UK.

Nitrate body burden was found to be higher in infants and children than in adults even when they consume the same or lesser amount of water. For example, at the drinking water standard of 50mg/l NO_3^-, nitrate body burden on infants was calculated as 4.8 mg/kg/day; 3.36 mg/kg/day for children (1-6 years); 1.71 mg/kg/day for children (7-12years); 0.72mg/kg/day for adolescents and 0.89 mg/kg/day for adults (19-70yrs). This suggests that nitrate body burden on infants was five times that of adults. For children (1-6years) the body burden was more than three times that of adults. At exposure concentration of 100mg/l NO_3, the body burden figures for infants, children (1-6years), children (7-12years), adolescents and adults were 9.8; 6.7; 3.5; 1.5 and 1.8 mg/kg/day respectively. This also suggest that nitrate body burden on infants is five times that of

adults. For children (1-6 years) the burden is also three times that of adults. The reason for the increased nitrate burden on infants and children relative to adults may be because of the small body weight of children compared to the adults. This suggests that infants and children are more sensitive to nitrate exposure in drinking water.

4. RISK CHARACTERISATION

The result of meta-analysis suggest that exposure to nitrates in drinking water is a risk factor for thyroid hypertrophy (goitre). Given that there is no register f or goitre in the UK, thyroid cancer was used as a proxy for goitre in the risk estimation. There is a close link between benign and malignant tumours as they share common aetiological factors (Leux & Guenel, 2010). Risk estimates suggest that the excess risk of thyroid cancer at nitrate levels below or equal to the drinking water standard of 50mg/l is 0.02 - 0.28. This is above the range ($1x10^{-6}$ to $1x10^{-5}$) considered as negligible by Health Canada (Health Canada, 2013). At the LOAEL of 69mg/l, the excess risk is 0.38. Assuming the linear model also applies to doses above 95mg/l (highest exposure dose in the source population), the population excess risk of thyroid cancer within the entire dose range of nitrates recorded in drinking water in East Anglia is 20 cases (13 per cent) out of the 154 cases calculated in a population of about 2.8million in 2014 and this is the number (20 cases) of thyroid cancer cases that would have been eliminated from the population in 2014 if there was no nitrates exposure in drinking water. The number of thyroid cancer cases reported for East of England (including East Anglia) in 2014 was 320 (ONS, 2016). High nitrate concentrations have been found in PWS than in public water, but the number of people in the study area exposed to PWS and therefore at a high risk of thyroid cancer is far less than the number of people exposed to public water where the risk is low. Although the risk of thyroid

cancer is higher among PWS users, it is not known if there are thyroid cancer clusters in East Anglia or other regions of the UK with high number of PWS.

The risk of thyroid cancer at low-doses i.e. below 50mg/l (drinking water standard) or below the LOAEL (69mg/l) supports the low-dose effect hypothesis and suggests that there is no threshold dose for nitrates on the thyroid gland. According to Vom Saal et al (2007) and Vandenberg et al (2012), substances or chemicals with known endocrine disrupting capabilities or potentials can have effects on biological systems at low doses which may result in some disease conditions in humans and the effect of these chemicals at high doses cannot be used to predict effects at low doses. The finding that there is no threshold dose for nitrates is consistent with reports by Berger et al (1997) and Gupta et al (1998), that exogenous nitrates via drinking water can increase the biological activity (including toxicity) of endogenously produced nitrate and as a result any threshold would already have been exceeded for this to occur, therefore there is no threshold for nitrates exposure. Also, as noted by Welshons et al (2003), exogenous chemicals (e.g. EDCs) can influence a process that is already ongoing and thus is already above threshold. This therefore contradicts the traditional toxicological assumption of threshold for endocrine responses to toxic chemicals like EDCs. There is no threshold for EDCs (Sheehan & vom Saal, 1997; Crews et al, 2000).

8.2 DISCUSSION

There is widespread nitrate contamination of drinking water sources especially PWS in some regions of the UK including East Anglia. In East Anglia, while the concentration of nitrates in public water between 2001 to 2010 was <0.1to 56.4mg/l, the concentration in PWS was 0.3 to 466mg/l. High nitrate levels have been found in PWS than in public supply especially in

agricultural areas due to the application of nitrogen based fertilizers to farm land and leaching of excess fertilizers to ground and surface water (DWI, 2016). PWS boreholes are more susceptible to contamination than public water boreholes because they are shallower compared to public water boreholes which are deeper. There is a strong relationship between nitrate concentrations in groundwater, the amount of nitrogen fertilisers applied to farm land and excess nitrate leaching from farm land (Grizzetti et al, 2011). Higher concentrations of nitrates have been found in groundwater than in surface water (Hunt et al, 2004; Lord et al, 2006) and in sandy soil than in clay soil (Hunt et al, 2004; Lord et al, 2006; Gupta et al, 2008). In groundwater, the presence of nitrates can remain for a very long time even after leaching from soil or farmland has stopped (Spalding and Exner, 1993; USGS, 1999).

Following a meta-analysis, there is a strong association between exposure to nitrates in drinking water and goitre. The relationship between exposure to nitrates in drinking water and goitre is linear and suggests that the risk of goitre increases as nitrate exposure increases. The risk of nitrate is more profound if there is decreased dietary iodine intake. Given that there is no register of goitre or benign thyroid tumour in the UK, thyroid cancer was used as a proxy for goitre in risk estimation. There is a close link between benign and malignant tumours as they share common aetiological factors (Leux & Guenel, 2010). Risk estimates suggests that the excess risk of thyroid cancer within the entire dose range of nitrates recorded in drinking water in East Anglia is 20 cases or 13 per cent of the 154 cases calculated in a population of more than 2.8million in 2014 and this is the number (20 cases) of thyroid cancer cases that would have been eliminated from the population in 2014 if there was no exposure to nitrates in drinking water. At nitrate concentration below and equal to the drinking water standard of 50mg/l, lifetime excess risk of thyroid cancer is 0.02 - 0.28. This is above the range ($1x10^{-6}$ to $1x10^{-5}$) considered as negligible by Health Canada (Health Canada, 2013). The excess risk of thyroid

cancer below and equal to the drinking water standard suggest that the relationship between nitrates and thyroid cancer is non-threshold. This suggests that every exposure to nitrates has some degree of effect on the thyroid gland. Given that nitrate is known to be produced endogenously for some biological activities (Jaffe, 1981; Walker 1995; Hsia, 1998) such as antimicrobial activity (Hibbs et al, 1987; Fang, 1997; Addiscott & Benjamin, 2004); vasodilation (Bjorne, 2005); neurotransmission (Garthwaite, 1991); immune regulation (Hibbs, 1991), any threshold would already has been exceeded for these effects to occur. Therefore the effect of exogenous nitrate exposure via drinking water which have been reported to increase the concentration of endogenously produced nitrate as well as its toxicity (Berger et al 1997, Gupta et al 1998) suggest a non- threshold effect. Although, there is no difference in tap- water intake between PWS and pubic water users in East Anglia, the amount of tap-water intake in the region is 0.8 to 1.2L/day. This is consistent with the amount of tap-water intake reported in the rest of the UK, 1.14 L tap-water per day (DWI 1996); 0.70 - 1.9 L/day (Hunter et al, 2004); 0.9-1.3 L/day (Hopkin & Ellis; 1980); 0.10 L - 1.55 L/day (Mons et al, 2007). On average, the amount of tap-water intake in the study area was 0.95 L/day for infants (≤ 1 year); 1.05 L/day for children (1-6years); 1.0 L/day for children (7-12years); 0.8 L/day for adolescents (13-18years) and 1.2 L/day for adults (19-70years). While nitrate body burden was found to be higher in infants and children than in adults even when they consume the same or lesser amount of water, none of the children aged 0-5 years in the study area were served with tap-water from PWS in households where the source of drinking water is usually PWS. This suggests that the marginal risk of thyroid cancer recorded in this age group may be related to exposure to nitrates from public water only. The ONS in its cancer records for 2014 for England (the latest thyroid cancer records) reported no thyroid cancer cases in children less than one year (<1 year). Only three thyroid cancer cases were recorded in children 1- 9 years and these cases were in females only

(ONS, 2016). Cases of thyroid cancer increased from age 10-14years and above according to ONS (2016).

The UK cancer statistics 2014 (ONS, 2016) suggest that thyroid cancer is the 19th most common cancer in males and the 16th most common cancer in females. In both sexes, it is the 19th most common cancer in the UK and accounted to about 1 per cent of all new cancer cases in 2014. In 2012, thyroid cancer was the 20th most common cancer in the UK and there is evidence of increasing incidence of the disease (Cancer Research UK, 2014; 2016). While increased detection of very small tumours due to widespread use of ultrasound; changes in thyroid cancer risk factors such as obesity and exposure to medical radiation may be contributory factors in the increasing thyroid cancer incidence, the finding from this study that exposure to nitrates in drinking water can result in excess risk of thyroid cancer especially in the event of moderate - to - mild iodine deficiency is consistent with the view that some environmental factors including diet, tobacco use, alcohol intake (Ron & Schneider, 2006) and nitrates(Pellegritti et al, 2013) may be contributing to the increasing thyroid cancer incidence. The continued prevalence of goitre in some iodine replete countries suggests that nitrate may be interfering with iodine uptake by the thyroid gland (Mukhopadhay et al, 2005). This highlights the need for a consideration of nitrate exposure as a risk factor for goitre and thyroid cancer in any public healthy strategy aimed at reducing the incidence of thyroid cancer in the UK. Such a strategy should include a well-designed epidemiological study to clarify the role of nitrate exposure in thyroid cancer with particular focus on PWS where the risk of thyroid cancer is higher than in public water supplies. The findings of a strong association with goitre in the meta-analysis and excess risk of thyroid cancer in the risk estimates support the recommendation by DWI (2014) for the inclusion of the safety of PWS in the Local Authority public health protection strategy especially in areas like East Anglia where more than 5per cent of the population rely on PWS for their drinking water.

In my view, the inclusion should be extended to all NVZs particularly those with PWS. Local authority health strategy is a requirement of the Health and Social Care Act (2012) which amended the Local Government and Public Involvement in Health Act (2007) which aimed to identify health needs and priorities in local areas and determine the required action to meet those needs in an evidence – based way (evidence –based public health).

Despite the increasing thyroid cancer incidence rate in the UK since the mid -1970s, the mortality rate from thyroid cancer has decreased and is now stable (Cancer Research UK, 2014). For example, between 1971-1973 and 2010-2012, the decline was 32 per cent in males and 58 per cent in females. In 2012, thyroid cancer accounted for 0.2 per cent of all cancer deaths in both males and females in the UK. The total number of thyroid cancer death in that year was 373 of which143 (38 per cent) were males and 230 (62 per cent) were females, giving male: female ratio of 6:10. The crude mortality rate was 0.5 thyroid cancer deaths for every 100,000 males and 0.7 thyroid cancer deaths for every 100,000 females (Cancer Research UK, 2014). The decline in mortality rate may be attributed to early diagnosis and treatment (Cancer Research UK, 2014). In Europe, whilst the incidence rate of thyroid has also increased in the last few decades, the mortality rate is stable at 0.4 per cent in 2012 (Cancer Research UK, 2014). In the USA, while the incidence rate increased by 5 per cent each year between 2002 and 2012; the mortality rate remained stable at 0.5 cases per 100,000 persons (The surveillance Epidemiology and End Result (SEER) Program of the National Cancer Institute, 2012). Unlike breast, colon-rectum, lung and prostate cancer whose mortality rate has declined in the USA in the last two decades, thyroid cancer mortality rate has not decreased despite early diagnosis and very effective treatment (SEER, 2012; Pellegriti et al, 2013). Absence of mortality decrease in spite of early diagnosis and treatment supports the evidence of increasing thyroid cancer incidence rate (Pellegriti et al, 2013).

8.2.1 IMPLICATIONS ON PUBLIC HEALTH POLICY

Although there are considerable uncertainties associated with the risk estimates derived in this study, the risk of thyroid cancer at nitrate doses below the WHO drinking water standard has implications on the current public health policy on nitrates in drinking water. The implication is that the current WHO and EU drinking water standard of 50mg/l NO_3^-, originally set to protect against infantile methaemoglobinemia is unlikely to protect against thyroid disorders including thyroid cancer and warrants a review by policy makers in order to protect public health. In a symposium on drinking water nitrate and health, the International Society for Environmental Epidemiology (ISEE) (2004), concluded that "the role for nitrate as a risk factor for cancer and adverse reproductive outcome must be thoroughly explored before changes to nitrate water quality standards are considered" (Ward et al, 2005: pg.1613). Similarly, the need for measures to reduce drinking water nitrate concentrations was recognised at a symposium in 2005 on Nitrogen Cycle and Human Health, although there was no consensus on the health risks of nitrates (van Grinsven et al, 2006). However, the findings from this study adds to the growing body of evidence that nitrate is a risk factor for thyroid cancer and supports the call for a review of the current drinking water standard for nitrates.

The findings from this study also have implications for EU policy on drinking water especially the EU Drinking Water Directive 98/83/EC and the UK PWS Regulation (2009). As already discussed in section 6.3.1, the EU drinking water directive as it relates to PWS only applies to large supplies i.e. all private water supplies providing water of $10m^3$/day or more (serving 50 or more persons) and /or part of a commercial or public activity (such as bed and breakfast establishments). It does not apply to small supplies (supplies providing water of less than $10m^3$/day or serving less than 50 persons) unless they are part of a commercial or public activity.

However, the UK government in implementing the Directive through the Private Water Supplies Regulation 2009 (previously PWS 1991) thought it wise to apply this Directive to small supplies (except those serving single dwellings) on the argument that, "the people consuming water and food prepared with water derived from these small supplies are entitled to the same level of health protection as the people served by large supplies and public water supplies" (DWI 2010, pg8). But given that 58 per cent of the 44,546 PWS in England serve single dwellings, the exclusion of this category of PWS from the mandatory risk assessment and monitoring regime as provided in the legislation can have implications on the health of the population (especially infants, children and pregnant women) living in such dwelling, putting them at risk of thyroid disorders (including thyroid cancer). These legislations therefore warrant a review.

Although the lifetime excess risk of 0.02 to 0.28 in this study at nitrate level ≤ 50mg/l relates to a moderate – to –mild iodine deficient population, the risk estimates may be higher in a population where iodine deficiency is severe. In countries such as the USA where about 22 per cent of private wells and 3 per cent of public wells in agricultural areas contain nitrates in excess of the USEPA drinking water standard of 45mg/l (US Geological Society cited in van Grinsven et al, 2006) and where about 15-20 per cent of households or about 15 million people obtain their drinking water from unregulated private well or borehole (USEPA cited in Rogan & Brady 2009), the findings from this study could have implications on public health protection policies and therefore warrant a review. In the USA, the Federal drinking water standard of 45mg/l NO_3 (10mg/l $N0_3$-N) only applies to public water supply; it does not apply to PWS (US GAO, 1997; Knobeloch, 2000). Although nitrate concentration in UK drinking water may be low when compared with other countries in Europe; the USA and developing countries, the findings from this study however highlight the potential health loss from exposure to nitrate in drinking water and the potential benefits of preventive measures.

In a discussion with two officials of Anglian Water Company while collecting data for this study (Personal Communication), it was noted that removing nitrate-ions from drinking water in order to comply with the current drinking water standard is expensive and adds to the operational costs of water companies. They suggested that raising the standard to about 100mg/l would reduce their operational cost and save their customers a lot of money on their water bill. This view has also been expressed (although in relation to infantile methaemoglobinemia) by L'hirondel et al, 2006), who argued that, "the societal costs of complying with the current MCL are growing, especially in rural communities least economically capable of shouldering the high cost per person of nitrate-ion removal" and suggested that "raising the drinking water standard for nitrates to 20ppm nitrate as nitrogen (equivalent to 88mg/lNO_3^-) would relieve many rural communities of a significant economic burden without adding appreciably to any known health risks" (L'hirondel et al, 2006: pg.459). However, given that risk estimates from this study suggest that the excess risk of thyroid cancer at nitrate level of 88mg/l (the level suggested by L'hirondel et al, 2006) is 0.43 or 43 per cent and at 100mg/l (the level suggested by Anglian Water) the risk is 0.46 or 46 per cent, raising the drinking water standard to either of these levels will pose significant risks to public health. The European Economic Community (EEC) proposed a maximum contaminant level (MCL) of 25mg/l (EEC, 1980; WHO, 1985b). This value has been reported as the critical concentration for adverse health effects of nitrate in drinking water (De Roos et al, 2003; Gulis et al, 2002). While at 25mg/l, the lifetime excess risk of thyroid cancer from this study is 0.15 or 15 per cent, a consideration of the EEC MCL may be a way forward.

While it is neither the aim nor the objective of this study to set a new drinking water standard for nitrates, a review of the current drinking water standard is necessary in view of the evidence from this study that the lifetime excess risk of thyroid cancer following exposure to nitrates in

drinking water below and equal to the drinking water standard of 50mg/l, is 0.02 to 0.28 (2 to 28 per cent). This is above the range ($1x10^{-6}$ to $1x10^{-5}$) considered negligible by Health Canada (Health Canada, 2013) and suggests that the current drinking water standard for nitrates, originally set to protect against infantile methaemoglobinemia is unlikely to protect against thyroid cancer. However, any downward review should also include a strategy to ensure that iodine intake in the population is optimum given that chronic exposure to low doses of nitrates in drinking water can result in thyroid disorders including goitre and malignant tumours in the event of iodine deficiency. Iodine is vital in the growth and development of the unborn child (Blazer et al, 2003) and iodine inhibition during pregnancy can affect the development of important organs in the foetus, especially the brain (Haddow et al, 1999; Blazer et al, 2003).

The suggestion that nitrates in drinking water can play a role in some reproductive and developmental outcomes including birth defects (Dorsch et al, 1984; Scragg et al, 1992; Croen et al, 2001), spontaneous abortion and stillbirth (Gelperin et al, 1975; Super et al, 1981; Aschengrau 1989; 1993; CDC, 1996); intra-uterine growth retardation and low birthweight (Tabacova et al, 1997;1998; Bukowski et al, 2001) may be as a result of iodine uptake inhibition by the thyroid gland and consequent low thyroid hormone production and this highlights the importance of optimum iodine intake in a population. However, further studies are needed to evaluate the relationship between exposures to nitrates in drinking water and adverse reproductive and developmental outcomes. Although infantile methaemoglobinemia is beyond the scope of this study, a review of the drinking water standard is important given that cases of this disease have been reported at nitrate levels below the drinking water standard of 50mg/l (Simon, 1962 cited in EWG (1996); Sattlemacher, 1964 cited in EWG (1996); Morbidity and mortality Weekly Report (MMWR), 1993). This information was not taken into consideration in

the various reviews of the drinking water standard by the USEPA (Carson, 1987) and the National Academy of Science (NAS, 1995).

8.2.2 REDUCTION OF NITRATE CONCENTRATIONS IN DRINKING WATER: COST - BENEFIT ANALYSIS

Reductions in nitrate concentration can be achieved in the short term by point- of- use or point-of- entry treatment. A point-of-use treatment involves the installation of a device, such as a filter at the point of entry of water to the household. It may be installed at the water supply tap or plumbed-in to the water supply pipe (Scottish Executive, 2006). These devices are usually used for the treatment of small volumes of water used for drinking or cooking. Devices used in the treatment of nitrate contaminated drinking water included reverse osmosis filters and anion exchange filters.

- Reverse osmosis filters employ reverse osmosis process in the removal of mostly organic and inorganic (e.g. nitrates) contaminants from drinking water.
- Anion exchange filters uses the anion or the cation exchange method to remove contaminants from drinking water and is best suited for the removal of nitrates from drinking water.

A reverse osmosis filter can waste a lot of water. It can also reduce water hardness and alkalinity to unacceptable levels. Anion exchange filters on the other hand require ‘regeneration’ and the softened water could contain elevated levels of sodium. In terms of cost, the reverse osmosis filter is medium- to- high; the anion exchange is low- to -medium, making the anion exchange filter the most popular filter used in short term nitrate treatment of drinking water in households (Scottish Executive, 2006).

In the long term, the most effective ways of reducing nitrate concentrations in drinking water are blending or mixing nitrate contaminated water with nitrate - free water; biochemical water treatment and installation of deeper water abstraction wells (Van Grinsven et al, 2010). While data is scarce on the cost of these measures and the potential benefits, available data suggests that the annual cost (in Euros €) of blending and biochemical treatment is €0.50/person/year in the UK and the Netherlands (when abstracting from a pre-existing well), with a potential health benefit of €0.70/person/year and €1.30/person/year for the UK and Netherlands respectively (Pretty et al, 2003; Van Beek et al, 2006 cited in Van Grinsven et al, 2010). The cost in Austria and Germany was €3.00/person/year when abstracting from a pre-existing well with a potential health benefit of €3.00/person/year and €5.00/person/year respectively (Ademsan et al, 2002 cited in Van Grinsven et al, 2010; Brandt 2002 cited in Van Grinsven et al, 2010). The cost of blending and treatment including installation of new boreholes or well was €15.00/person/year in the USA (Van Grinsven et al, 2010). Given persistent nitrate failures in drinking water especially PWS in many parts of East Anglia, it is not known whether this data on potential health gains will persuade some individuals and water companies to invest in water treatment and/- or new infrastructure (e.g. deeper wells) as the cost may initially appear to be higher than the potential health gains. While some residents in a Local Authority with high nitrate concentrations in their PWS have rejected (for unspecified personal reasons) advice from environmental health officers to connect to public supplies, some have expressed willingness to connect to the public supply where nitrate concentrations are much lower, but unfortunately the cost of this undertaking is prohibitive, as some houses are located too far from the nearest public water supply location and the Water Companies concerned were not willing to bear the cost of the connection. While people have often made enquiries about grants to enable them connect to the public supply, no such grant is currently available. The government could through the Water Companies or Local Authorities make such grants available to enable those willing to migrate from PWS (where

nitrate levels are sometimes high) to a public supply to be able to do so. The willingness to a connect to public supply in order to have access to drinking water with low nitrates concentrations below the WHO drinking water standard is consistent with earlier findings by Hanley (1990) that households in East Anglia in 1989 expressed willingness to pay for drinking water with nitrate concentrations not exceeding the EU drinking water standard.

The use of manure and fertilisers in crop production is a major source of nitrates in ground and surface water especially in agricultural areas such as East Anglia and reduction in the quantity of fertilisers and manure applied to crops is critical in reducing the concentration of nitrates in drinking water. The aim of such reduction to prevent excess fertilizers not used up by crops from leaching into groundwater and surface water used for drinking water and is the objective of the EU Nitrate Directive 91/676/EEC. However, despite measures in place to reduce excessive input of fertilizers to crops especially in farm lands near to water courses, drinking water sources in most European countries (including the UK) have continued to experience excessive nitrate pollution (Grizzetti et al, 2011). Van Grinsven et al (2010) estimated that the health cost associated with nitrates leaching to water sources is €0.15/kg of N-fertilizer while the net benefit of fertilizer use is €1.8/kg of N-fertilizer. This suggests that the health cost of fertilizer leaching into drinking water sources reduced the net benefit of fertiliser use in crop production by about 10 per cent. In some countries in north-western Europe where crop yield is reported to be close to their maximum or in southern and eastern Europe where yields are limited by water shortages, the health cost associated with nitrate leaching estimated as €0.5/kg/N-fertiliser is almost the same as the net benefit (€0.6/kg/N-fertiliser) of fertiliser use (Van Grinsven et al, 2010). This suggests that reductions in the quantity of fertilizers applied to crops would help to reduce the health costs of leaching and maximise the net benefits of fertilizer use. While nitrate concentrations in aquifers in some western European countries has not decreased but are rather

stable, there are concerns that concentrations in Eastern European countries will continue to increase as these countries intensify agricultural activities and fertiliser use (Grizzetti et al, 2011). While agricultural practices and other anthropogenic activities will continue to impact on drinking water sources in Europe even in the coming years, it is not known what and how long it will take to restore the quality of European waters (Grizzetti et al, 2011).

Given widespread nitrate contamination of drinking water especially PWS in parts of the UK and the EU, the potential for widespread exposure in the population makes the review of the drinking water standard an imperative for public health protection. In a review of data from 12 of the 15 oldest EU Member States in the course of implementing the EU Drinking Water Directive 98/83/EC, van Grinsven et al (2006) and van Grinsven et al, (2010) estimated that about 23 million people (6.5 per cent of the total population) who obtained their drinking water from groundwater are exposed to drinking water with nitrate concentration exceeding 25mg/l. About 8 million people (3 per cent) of this population are exposed to groundwater with nitrate concentration exceeding the drinking water standard of 50mg/l, about 5 per cent were exposed to groundwater with nitrate concentrations exceeding 25mg/l. When this data from groundwater was combined with data from European surface water and population density, it was estimated that about half of the EU27 population live in areas with nitrate concentrations higher than 25mg/lNO_3 while about one fifth (20 per cent) live in areas with concentrations higher than 50mg/lNO_3, although there may be country variations (Grizzetti, 2011).

Although causality has not been firmly established in this study between exposure to nitrates in drinking water and thyroid disorders including thyroid cancer, the need for a review of the current drinking water standard is based on the precautionary principle. The precautionary principle states that, “when an activity raises threats of harm to human health or the environment,

precautionary measures should be taken even if some cause and effect relationship are not fully established scientifically" (Wingspread Conference 1998). As noted by Hill (1965), lack of consensus or perfect evidence of causality "does not confer upon us a freedom to ignore the knowledge we already have, or to postpone the action that it appears to demand at a given time" (Hill 1965: p300). The precautionary principle has been proposed as an approach in public health decision making in the face of uncertainty (O'Riordan & Cameron, 1994; Kriebel et al, 2001; Gochfeld, 2003; USEPA, 2004). It is used to articulate public health policy or take regulatory action in the absence of conclusive scientific evidence demonstrating harm (O'Riordan & Cameron, 1994; Massachusetts Precautionary Principle Project (MPPP) 1999; Neutra & Delpizzo, 2002; Pless, 2003; Gochfeld 2003). The approach encourages taking measures or actions, including interim actions for public health protection before additional information becomes available (Kriebel et al, 2001; Gochfeld 2003). The alternative to taking action, 'more research', or "wait- and- see" anticipates conclusive scientific evidence for causality which may take some considerable time to be produced and may therefore delay action when the consequences of inaction or delay may be serious (Gochfeld, 2003). Although the precautionary principle concept has been criticised because of variability in interpretation, it offers the prospects of real progress towards improved public health decision-making (Sexton, 2006).

While low dietary iodine intake can exacerbate the effect of nitrates on the thyroid gland, iodine prophylaxis in the form of salt iodisation or use of iodised salt in bread making can help to maintain adequate iodine in the body. But, as noted by Wilson (2010), lowering the concentration of nitrates in drinking water is a more effective approach in combating the effect of human exposure to iodine inhibitors than increasing total iodine intake. However, given that the cost of salt iodisation in order to deal with the effects of iodine deficiency (US$ 0.02 - €0.05/child/year) is less than the cost of water treatment and blending (€0.5 - €3.00/person/year)

to ensure that nitrates in drinking water does not exceed 25mg/l, the health benefits of both measures outweigh their costs and can be integrated into any public health policy on nitrates, especially in areas where there is widespread nitrate pollution of drinking water and the potential for chronic exposure. While it is beyond the scope of this study to estimate the economic loss from thyroid cancer as a result of exposure to nitrates in drinking water, Van Grinsven et al (2010) estimated that a 3per cent rise in colon cancer incidence as a result of exposure to nitrates in drinking water above 25mg/l corresponds to an economic loss of €2.90/person/year. Assuming the economic loss of colon cancer also applies to other types of cancer like thyroid cancer, then the 13 per cent excess thyroid cancer cases in East Anglia attributed to exposure to nitrates in drinking water will correspond to an economic loss of €12.60/person/year; this could be eliminated if there were no nitrates in drinking water.

8.3 CONCLUSION

Exposure to nitrates in drinking water is associated with the risk of thyroid cancer. Of the 154 thyroid cancer cases calculated for East Anglia in 2014 from a population of 2,849,918; the excess risk is 20 cases (13 per cent) and this would have been eliminated if there was no exposure to nitrates in drinking water. The number of thyroid cancer recorded in East of England (including East Anglia) by ONS in 2014 was 320. At nitrate levels below and equal to the drinking water standard of 50mg/l, the lifetime excess risk was 0.02 to 0.28. This is above the range considered negligible by Health Canada. The public health policy implication of this finding is that the current drinking water standard of 50mg/l, originally set to protect against infantile methaemoglobinemia is unlikely to protect against thyroid cancer and warrant a downward review. Also, the EU Drinking Water Directive 98/83/EC which excluded small PWS and the UK PWS Regulation 2009 which excluded small PWS serving single dwellings from the

mandatory risk assessment which applies to large supplies warrant a review given that the risk of thyroid cancer is higher among PWS users where nitrate levels are higher. Given that 58 per cent of the 44,546 PWS in England serving single dwellings are excluded from the five years mandatory risk assessment and monitoring regime as provided in the PWS Regulation (2009), a review of this policy is necessary in order to protect the health of the public in such households from the risk of thyroid cancer. Any downward review should also include a strategy to ensure that iodine intake in the population is optimum given that chronic exposure to low doses of nitrates in drinking water can result in thyroid disorders including goitre and thyroid cancer in the event of dietary iodine deficiency. In countries such as the USA where PWS are not regulated (the drinking water standard of 45mg/l only applies to public supplies), a review of this policy is very important given that the risk of thyroid cancer and thyroid disorders is higher among PWS users than public supply users. Also, in developing countries where PWS are unregulated, the evidence of thyroid cancer risk from this study as a result of nitrate exposure in drinking water can help to bring this category of supplies into the public health agenda.

A review of the current policy on nitrate should include a consideration of lowering the drinking water standards. While it is neither the aim nor the objective of this study to set a new drinking water standard for nitrates, the EEC recommended guideline value or MCL is 25mg/l may be a way forward. This value, which has been reported as the critical concentration for adverse health effects for nitrate and below which, the risk of thyroid cancer is low, is associated with lifetime excess risk of 0.15 or 15%. Reduction of nitrate concentrations in drinking water can be achieved by blending nitrate contaminated water with nitrate free water; biochemical treatment of water and/or reducing the amount of fertilisers or manure applied to crops. Reduction in the amount of fertilisers or manure applied to crops can help prevent leaching of excess fertilisers to ground or surface waters used for drinking water purposes and this is the objective of the EU Nitrate

Directive. Whilst the health benefits of blending and biochemical treatment to prevent nitrate concentrations exceeding 25mg/l in drinking water have been judged to far outweigh the cost, the health cost associated with nitrate leaching to drinking water sources can impact on the net benefit of fertiliser use for crop production.

The health impact of the 13 per cent excess thyroid cancer cases attributed to nitrates in drinking water in the study population could translate to an economic loss of €12.60/person/year. While the cost of salt iodisation to deal with the effects of iodine inhibition is less than that of water blending to reduce nitrate concentration in drinking water, both measures can be incorporated in any public health policy designed to reduce the health impact of nitrate exposure. This is important because of the evidence of the persistence of goitre in some countries that have successfully implemented salt iodisation. Iodisation of salt is safe, simple, cheap, self- financing and cost - effective and there is no significant price differential between iodised and non-iodised salt. It has the benefit of improving maternal iodine status and cognitive function in new born babies and school-age children as well as school performance. Also, there are benefits in terms of goitre prevalence and hyperthyroidism; and it provides alternative sources of iodine to people who may be intolerant or allergic to dairy milk. However, a national survey of iodine status of the UK population is necessary before the implementation of any iodine prophylaxis programme given that the evidence of moderate –to mild iodine deficiency in the UK is only based on studies on children of school age. This is important in order to avoid the risk of iodine induced hyperthyroidism as a result of excess iodine intake. Given that iodine is essential for the maintenance and outcome of pregnancy, women of child-bearing age should be encouraged to take vitamins fortified with iodine if they are planning on getting pregnant. Also, iodine fortified vitamins must be made essential for pregnant and lactating women in order to improve the outcome of pregnancy and improve cognitive function in new-born babies.

Although causality was not firmly established between nitrate exposure in drinking water and thyroid cancer, a role for nitrates in the aetiology of thyroid disorders including goitre and thyroid cancer is biologically plausible and the measures outlined in this study to mitigate the health impact of nitrates following exposure in drinking water can help to improve the health of the public as a precaution. While the results of epidemiological studies on thyroid disorders including thyroid cancer are inconsistent, more and improved epidemiological studies are necessary in order to clarify the possible role of nitrates in thyroid cancer aetiology given the increasing incidence of the disease in the UK and other countries around the world without an identifiable cause.

8.4 RECOMMENDATIONS

- Review the current drinking water standard for nitrates and consider lowering the standard in view of the evidence of risk of thyroid cancer at nitrates concentration below and equal to 50mg/l.

- Evaluate existing and new water treatment technologies for nitrates in drinking water with a view to effective removal of nitrate ions from drinking water.

- Review EU Directive 98/83/EC on the quality of water used for human consumption with a view to extending the Directive to all PWS irrespective of the size and the number of people served.

- Review the UK Private Water Supply Regulation (2009) and extend the 5 years compulsory risk assessment and monitoring regime to all PWS including those serving single dwellings, given that this type of supply constitutes the majority of PWS in the UK but they are currently exempt from the compulsory risk assessment and monitoring regime that applies to all other supplies.

- A survey of the iodine status of the UK population especially in regions such as East Anglia where there is widespread nitrate contamination of drinking water sources. Given that no national survey has been undertaken to monitor the iodine status of the UK population since the 1940s, the survey will help to bring iodine back on to the public health agenda and encourage consideration the implementation of iodine prophylaxis in the population.

- Advice to pregnant women and lactating mothers (especially those served by PWS) should include information on the adverse health effects of nitrates in drinking water and the need to avoid water with high concentration of nitrates during critical periods.

8.5 FURTHER RESEARCH

- Well-designed epidemiological studies are required to clarify the role of nitrates in drinking water and thyroid cancer. The studies should focus more on PWS given the vulnerability of this type of drinking water sources to nitrate contamination. Such studies should consider the amount of iodine intake in the study population; the actual nitrate concentration in the drinking water and the amount of tap-water intake per day. Also, the study should include population with long- term exposure including those on public

supplies. Drinking water contaminants that occur alongside nitrates e.g. perchlorate, thiocyanate and pesticides should also be evaluated.

- Studies are required to investigate whether there are thyroid cancer clusters in regions of the UK where there are high numbers of PWS.

- Further investigation is required to confirm the relationship between nitrates in drinking water and thyroid disorders, including thyroid cancer. Such studies should determine whether the relationship is threshold or non-threshold; monotonic or non- monotonic.

- Studies are required on the economic loss from thyroid cancer including the cost of treatment.

- Studies are required on the reproductive and developmental outcomes as a result of exposure to nitrates in drinking water. Such studies should evaluate the relationship between exposure to nitrates in drinking water and preterm birth; spontaneous abortions; intrauterine growth retardation; malformations of the central nervous system and low birthweight.

REFERENCES

Abernathy C, Roberts W (1994): Risk assessment in the Environmental Protection Agency. Journal of Hazardous Substances. **39**, 135-142

Adedapo KS, Fadiji IO, Orunmuyi AT, Ejeh JE, Osifo BO 2012): High default rate in thyroid cancer management in Ibadan, Nigeria: a need for health insurance. Africa Journal of Medical Sciences, Suppl: **14**: 105-9 (2012).

Addiscott T and Benjamin N (2004): Nitrate and human health. Soil Use and Management **20**, 98-104.

Aggleton P (1995): Health: Routledge, London and New York (1995).

Akinbami LJ, Lynch CD, Parker JD, Woodruff TJ (2010): The association between childhood asthma prevalence and monitored air pollutants in metropolitan areas, United States, 2001–2004. Environmental Research **110**:294–301

Alexander W, Wolff J (1966): Thyroidal iodide transport: relation between transport, goitrogenic and antigoitrogenic properties of certain anions. Endocrinology **78**,581-590

Alexander NM (1962): Assay procedure for thyroid peroxidase. Analytical Biochemistry **4**: 341–345.

Alexandrov V.A, Dzhioev FK (1977): Inhibitory effect of a number of substances on manifestation of the embryotoxic and teratogenic effects of methylurea and sodium nitrate. Biology-Med 1977: **83** (1): 75-77.

Allen L, Bruno de Benoist, Omar D, Hurrell R (2006): Guidelines on food fortification with micronutrients. World Health Organization and Food & Agricultural Organization of the United Nations, Geneva, 2006

Al-Sa'doni H and Ferro A (2000): S-Nitrosothiols: a class of nitric oxide-donor drugs: Clinical Science (London) **98,** 507-520

Alyea RA, Watson CS (2009): Differential regulation of dopamine transporter function and location by low concentrations of environmental oestrogens and 17-estradiol. Environmental Health Perspective **117**:778–783.

Andersson M; de Benoist B; Darnton-Hill I; Delange F (2007a): Iodine deficiency in Europe: A continuing public health problem. World Health Organisation, 2007.

Armstrong B.G (1998): Effect of measurement error on epidemiological studies of environmental and occupational exposures. Occupational Environmental Medicine, **55** (10):651-656.

Andersson, M.; de Benoist, B.; Delange, F.; Zupan, J (2007b): Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: Conclusions and recommendations of the Technical Consultation. Public Health Nutrition. **10**, 1606–1611.

Andersson M, Karumbunathan V, Zimmermann MB (2012): Global iodine status in 2011 and trends over the past decade. Journal of Nutrition. **142(4)**:744-50.

Aoki, Y., Belin, R.M., Clickner, R., Jeffries, R., Phillips, L. and Mahaffey, K.R. (2007): Serum TSH and total T4 in the United States population and their association with participant characteristics: National Health and Nutrition Examination Survey (NHANES 1999–2002). Thyroid, **17**(12): 1211–1223.

Arbuckle TE, Sherman G.J, Corey P.N (1988): Water nitrate and CNS birth defects: a population based case controlled study. Archieves of Environmental Health, **43**: 162-7.

Aschebrook-Kilfoy B, Ward M, Sabra M, Devesa S (2011): Thyroid cancer incidence patterns in the United States by histologic type, 1992-2006. Thyroid; **21(2):**125-34.

Aschengrau A, Zieler S, Cohen A (1989): Quality of community drinking water and the occurrence of spontaneous abortion. Archives of Environmental Health **44**: 283-290. 1989.

Aschengrau A, Zierler S, Cohen A (1993): Quality of community drinking water and the occurrence of late adverse pregnancy outcomes. Archieves of Environmental Health **48:**105–113. 1993.

ATSDR (2001): Agency for Toxic Substances and Disease Registry. Nitrate and Nitrite Toxicity. USA Dept of Health and Human Services. ATSDR Publication No. ATSDR-HE-CS-2002-0007.

Avery AA (1999): Infantile methaemoglobinemia: re-examining the role of drinking water nitrates. Environmental Health Perspective, **107**, No 7: 583-586.

AWWARF (1995): American Water Works Association Research Foundation. Nitrification occurrence and control in chlorinated water system. American Water Works Association Research Foundation Denver, Colorado.

Axelso O (1985). Epidemiological methods in the study of spontaneous abortions: Sources of data, methods and sources of error. IN: Occupational hazards & reproduction. Eds. Hemminki K, Vaino H, Sorsa M Pg. 231 –236. Hemisphere Press, Washington DC 1985.

Ayebo A, Kross B, Vlad M, Sinca A (1997): Infant methemoglobinemia in the Transylvania region of Romania. International Journal of Occupational Environmental Health **3(1):**20–29.

Bahadoran Z; Mirmiran P, Ghasemi A, Kabir A, Fereidoun Azizi P; Hadaegh F (2015): Is dietary nitrate/nitrite exposure a risk factor for development of thyroid abnormality? A systematic review and meta-analysis, Nitric Oxide **47,** 65–76

Baghurst PA, McMichael AJ, Slavotinek AH et al. (1991): A case – control study of diet and cancer of the pancreas. American Journal of Epidemiology, **134**:167–179.

Balasubramaniam S, Elaine Ron E, Gridley G, Schneider A, Brenner A (2012): Association between Benign Thyroid and Endocrine Disorders and Subsequent Risk of Thyroid Cancer among 4.5 Million U.S. Male Veterans. Journal of Clinical Endocrinology and Metabolism. **97**(8): 2661–2669

Ball D (2006): Environmental Health Policy: Understanding public health. Open University Press 2006

Baker M; Venugopal K; Howden-Chapman P (2011): Household crowding and tuberculosis. In: Environmental burden of disease associated with inadequate housing. Methods for quantifying health impacts of selected housing risks in the WHO European Region, Pg. 57- 80. Edited by: Braubach M, Jacobs D.E, Ormandy D.

Barbone F, Valent F, Brussi V, Tomasella L, Triassi M, Di Lieto A, Scognamiglio G, Righi E, Fantuzzi G, Casolari L, Aggazzotti G (2002): Assessing the exposure of pregnant women to drinking water disinfection byproducts. Epidemiology: **13 (5)**:540–544.

Barden HS & Kessel R (1984): The costs and benefits of screening for congenital hypothyroidism in Wisconsin. Social Biology **31**: 185–200.

Barker PR, Schopfer FJ, Sweeney S, Freeman B.A (2004): Red cell membrane and plasma linoleic acid nitration products: synthesis, clinical identification and quantification. Proceedings of National Academy of Science USA **101**:11577-82.

Barr DB, Olsson AO, Wong LY, Udunka S, Baker SE, Whitehead RD, Magsumbol M, Williams B, Needham L (2010): Urinary concentrations of metabolites of pyrethroid insecticides in the general U.S. population: National Health and Nutrition Examination Survey 1999–2002. Environmental Health Perspective 118:742–748.

Barrett JH, Parslow RC, McKinney PA et al. (1998). Nitrate in drinking water and the incidence of gastric, oesophageal, and brain cancer in Yorkshire, England. Cancer Causes Control, **9**:153–159.

Barrett et al (1999): Marker species for identifying urban groundwater recharge. A review and case study in Nottingham UK.

Barnes G, Dourson M (1988): Reference Dose (RfD): Description and use in health risk assessment. Regulatory Toxicology & Pharmacology. **8**, 471- 486

Bartholomew B.A, Caygill C, Darbar R, Hill M J (1979). Possible use of urinary nitrate as a measure of total intake. Proceeding of the Nutrition Society. **38**,124 A.

Bartholomew B.A, Hill MJ, Hudson MJ, Ruddell WS, Walters CL (1980). Gastric bacteria, nitrate, nitrite and nitrosamines in-patients with pernicious anaemia and in-patients treated with cimetidine. IARC Scientific Publications, **31**, 595-608.

Bartholomew B.A, Hill M J (1984). The Pharmacology of dietary nitrate and the origin of urinary nitrate. Food Chemistry and Toxicology **22,**789-694.

Bartsch H, Ohshima H, Pignatelli B, Calmels S (1992): Endogenously formed N-nitroso - compounds and nitrosating agents in human cancer etiology. Pharmacogenetics, **2**:272–277.

Bartsch H, Ohshima H, Pignatelli B (1998): Inhibitors of endogenous nitrosation. Mechanism and implications in human cancer prevention. Mutation Research (1998) **202**:307-324

Bates J, baker M, Guerra Jr. R, Harrison D (1991): Nitric oxide generation from nitroprisside by vascular tissue. Evidence that reduction of the nitroprusside anion and cyanide loss are required Biochem. Pharmacology **42** (suppl), s157-165

Bath S, Walter A, Taylor A, Rayman M (2008): Iodine status of UK women of childbearing age. Journal of Human Nutrition and Diet **21**:379–380.

Bath S, Wright J, Taylor A, Walter A, Rayman M P (2010): Iodine deficiency in pregnant women living in the South-East of the UK. Proceedings of Nutrition Soceity, 2010; **69**.

Bath S.C, Steer C.D, Golding J, Emmett P, Rayman M.P (2013): Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). The Lancet: **Volume 382**, Issue 9889, pages 331 - 337, 27 July 2013.

Beaglehole R, Bonita R, Kjellstrom T (1993): Basic Epidemiology (pg 1-2). World Health Organisation 1993.

Becker DV, Braverman LE, Delange F, Dunn JT, Franklyn JA, Hollowell JG, Lamm SH, Mitchell ML, Pearce E, Robbins J, Rovet JF (2006): Iodine supplementation for pregnancy and lactation-United States and Canada: recommendations of the American Thyroid Association. Thyroid.**16**:949–951.

Beckman JS, Ischiropoulos H, Chen J (1991): Nitric oxide as a mediator of superoxide dependent injury. In: Oxidative damage and repair. Edited by Davies KTA. Pergammon: Oxford; 1991:251-255.

Below, H., Zollner, H., Volzke, H. and Kramer, A. (2008): Evaluation of nitrate influence on thyroid volume of adults in a previously iodine-deficient area. International Journal of Hygiene and Environmental Health, **211(1–2):** 186–191.

Benichou J (1991): Methods of adjustment for estimating the attributable risk in case-control studies: a review. Statistics in Medicine. **10**:1753-1773.

Benjamin N, O'Driscoll F, Dougall H, Duncan C, Smith L, Golden M, McKenzie H (1994): Stomach NO synthesis. Nature 368—502.

Beresford SA (1985). Is nitrate in the drinking water associated with the risk of cancer in the urban UK? International Journal of Epidemiology, 14:57–63.

Berger H, Moison R (1986): Patho-physiology of respiratory distress syndrome: In: 14th Recent Advances in Paediatrics (Eds. David T) pp 117-119

Bergman I, Hirsch RP, Fria TJ, Shapiro SM, Holzman I, Painter MJ (1985): Causes of hearing loss in the high-risk premature infant. Journal of Paediatrics (1985), **106**:95-101.

Bern H. A. (1992): The fragile fetus: In: Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection (Colborn T, Clement C, (eds). Vol. 21 Princeton, NJ: Princeton Scientific Publishing, pp9–15.

Bernhard C, Carbiener R, Cloots A.R (1992). Nitrate pollution of groundwater in the Asation Plain France – a multidisciplinary study of an agricultural area. The Central Reid of the I 11 River: Environmental Geology and Water Sciences **20**, 125-137.

Berresheim H, O'Herlihy C (2013): Maintenance of iodine intake. Thyroid Research 2013, **6 (suppl.2)** A52, 1-3.

Bilau M, Matthys C, Baeyens W, Bruckers L, De Backer G, Den Hond E, Keune H; Koppen G, Nelen V, Schoeters G, Van Larebeke N, Willems J, De Henauw S (2008): Dietary exposure to dioxin-like compounds in three age groups: results from the Flemish environment and health study. Chemosphere **70(4):**584–592.

Bingham SA. (1999): High-meat diets and cancer risk. Proceedings of Nutritional Soceity **58**:243–248.

Bingham SA, Hughes R, Cross AJ. (2002): Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. Journal of Nutrition **132**:3522S–3525S.

Bingham SA, Pignatelli B, Pollock JR, Ellul A, Malaveille C, Gross G, et al. (1996): Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis **17**:515–523.

Birnbaum (2012): Environmental chemicals, evaluating low-dose effects. Environmental Health Perspective. **Vol. 120: No. 4.** Pg. A143-144.

Bjorne H (2005): The nitrite ion: Its role in vasoregulation and host defences. Karolinska Institute Sweden 2005

Blazer S, Moreh-Waterman Y, Miller-Lotan R, Tamir A, Hochberg Z (2003): Maternal hypothyroidism may affect fetal growth and neonatal thyroid function. Obstetric & Gynocology**102**:232–241.

Blomberg M, Feldt-Rasmussen U, Andersen KK, Kjaer SK (2012): Thyroid cancer in Denmark 1943-2008, before and after iodine supplementation. International Journal of Cancer 2012; **131(10):**2360-6.

Bloomfield RA, Welsch CW, Garner G, Muhrer ME (1961): Effect of dietary nitrate on thyroid function. Science 1961; **134**:1690.

Bloomfield RA, Welsch CW, Garner GB, Muhrer ME (1962): Thyroid compensation under the influence of dietary nitrate. Proc Soc Exp Biol Med. 1962; **111**:288–290.

Blount BC, Valentin-Blasini L, Mauldin JP, Pirkle JL, Osterloh JD (2007): Perchlorate Exposure of the U.S. Population, 2001–2002. J Exposure Science &Environmental Epidemiology **17**:400–407.

Blount BC, Rich DQ, Valentin-Blasini L, Lashley S, Ananth CV, Murphy E, Smulian JC, Spain BJ, Barr DB, Ledoux T, Hore P, Robson M (2009): Perinatal exposure to perchlorate, thiocynate and nitrate in New Jersey mothers and newborns. Environmental Science & Technology. **43(19):** 7543-7549.

Blount B, Alwis K, Jain B, Solomon B, Morrow J, Jackson W (2010): Perchlorate Nitrate, and Iodide intake through tap water. Environmental Science & Technology, **44** 9564-9570 (2010).

Blowers L, Preston-Martin S, Mack WJ (1997): Dietary and other lifestyle factors of women with brain gliomas in Los Angeles County (California, USA). Cancer Causes Control, **8**:5–12.

Boeing H, Frentzel-Beyme R, Berger M, Berndt V, Gores W, Korner M, Lohmeier R, Menarcher A, Männl HF, Meinhardt M (1991): Case–control study on stomach cancer in Germany. International Journal of Cancer, **47**:858–864.

Boeing H, Schlehofer B, Blettner M, Wahrendorf J (1993): Dietary carcinogens and the risk for glioma and meningioma in Germany. International Journal of Cancer **53**:561–565.

Boeing H. (1991): Epidemiological research in stomach cancer: progress over the last ten years. J Cancer Res Clinical Oncololgy **117**:133–143.

Bogovski P, Bogovski S (1981): Animal species in which N-nitroso compounds induce cancer. International Journal of Cancer **1981, 27**:471- 474.

Bois F, Boudet C, Diack C (2006): Uncertainty in risk assessment: INTARESE Uncertainty concept report. Deliverable 11 (Unpublished).

Bonnell A (1995). Nitrate concentrations in vegetables. In: Health aspects of nitrates its metabolites (particularly nitrites). Proceedings of an international workshop. Bilthoven Netherlands 8-10 November 1994 pg 11-20. Council of Europe Press, Strasbourg.

Bournaud C, Orgiazzi JJ (2003): Iodine excess and thyroid autoimmunity. Journal of Endocrinological Investigation, 2003, **26**:49–56.

Bove F, Fulcomer M, Klotz J, Esmart J, Dufficy E, Savrin J (1995): Public Drinking Water Contamination and Birth Outcomes. American Journal of Epidemiology 1995; **141**: 850-862.

Bove F, Shim Y, Zeitz P (2002): Drinking Water Contaminants and Adverse Pregnancy Outcomes: A Review. Environmental Health Perspectives 2002; **110 (Supp1):** 61-74

Bove FJ; Fulcomer M.C; Klotz J B; Esmart J; Dufficy E.M; Zagraniski R; Savrin JE (1992): Population-based Surveillance and Etiological Research and of Adverse Reproductive Outcomes and Toxic Waste Report on Phase IV – A: New Jersey department of Health 2002.

Bradshaw, J. (1972): The concept of need. New Society, **30**, 640-643.

BrantsæterA L, Abel M H, Haugen M, Meltzer H M (2013): Risk of Suboptimal Iodine Intake in Pregnant Norwegian Women. Nutrients 2013, ***5*(2),** 424-440.

Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM, Lanphear BP (2009): Prenatal bisphenol A exposure and early childhood behavior. Environmental Health Perspective **117**:1945–1952.

Braun JM, Hauser R. (2011): Bisphenol A and children's health. Current Opinion Pediatrics **23(2):**233–239.

Braverman L.E, He X, Pino S, Cross M, Magnani B, Lamm S, Kruse M, Engel A, Crump K, Gibbs J (2005): The effect of perchlorate, thiocynate and nitrate on thyroid function in workers exposed to perchlorate long – term. Journal of Clinical Endocrinololy & Metabolism **90**: 700-706.

Brender JD, Olive JM, Felkner M, Suarrez L, Marckwardt W, Hendrick K.A (2004): Dietary nitrites and nitrates, nitrosatable drugs and neural tube defects. Epidemiology **15 (3):** 330 -336.

Brender JD, Weyer PJ, Romitti PA, Mohanty BP, Shinde MU, Vuong AM, Sharkey JR, Dwivedi D, Horel SA, Kantamneni J, Huber JC Jr, Zheng Q, Werler MM, Kelley KE, Griesenbeck JS, Zhan FB, Langlois PH, Suarez L, Canfield MA; National Birth Defects Prevention Study (2013): Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the national birth defects prevention study. Envornmental Health Perspective, **121(9):**1083-9.

Brenner H, Savitz DA, Jöckel KH, Greenland S (1992): Effects of nondifferential exposure misclassification in ecologic studies. American Journal of Epidemiology 135(1): 85-95.

Bricker T, Jefferson LS, Mintz A (1983): Methemoglobinemia in infants with enteritis. Journal of Pediatrics **102**:161–162.

Briggs D (2008): A framework for integrated environmental health impact assessment of systemic risks. Environmental Health, **7:** 61, (2008).

British Medical Association: http://bma.org.uk/news-views-anlysis/news/2015/january/local-authorities. Accessed 2/3/15.

Brown, J.L. (1999). N-Nitrosamines. Occupational Medcine, **14**(4): 839–848.

Brownson RC, Gurney JG, Land GH (1999): Evidence-Based Decision Making in Public Health. Journal of Public Health Management Practice, 1999, **5(5)**, 86–97.

Brownson RC, Fielding JE, Maylahn CM. (2009): Evidence-based public health: a fundamental concept for public health practice. Annual Review of Public Health **30**:175–201.

Brownson RC, Fielding JE, Maylahn C. (2013): Evidence-based Decision Making to Improve Public Health Practice. Frontiers in Public Health Services & System Research, **Vol. 2: No 2**.1-9.

Bruce GM, Peterson MK, Pleus RC (2004): Comparative contribution of perchlorate and anti-thyroid agents in American diets to iodine uptake inhibition. Paper presented at the 32nd

Propellant Developement & Characterisation subcommittee and 21st Safety & Environmental Protection subcommittee Joint Meeting Seatle, WA, 26 July 2004.

Bruce G, Corey L, Mandel J, Pleus R (2013): Urinary nitrate, thiocyanate, and perchlorate and serum thyroid endpoints based on NHANES 2001 to 2002. Journal of Occupational and Environmental Medicine, **55**; 52–58.

Bruchovsky N, Lesser B, Van Doorn E, Craven S (1975): Hormonal effects on cell proliferation in rat prostate. Vitam Horm **33**:61–102.

Bruningfann CS and Kaneene JB (1993): The effects of Nitrate, nitrites and N-nitroso compounds on animal health. Veterinary and Human Toxicology, **35:** 237 –253.

Bryan N; Calvert J.W; Elrod J.W; Gundewar S; Ji S Y; Lefer D. J (2007): Dietary nitrite supplementation protects against myocardial ischemia-reperfusion injury. Proceedings of National Academy of Science USA **104**:19144–19149.

Bruzzi P, Green S.B, Byar D.P, Brinton LA, Schairer C (1985): Estimating the population attributable risk for multiple risk factors using case-control data. American Journal of Epidemiology. **122:**904-914.

Buiatti E, Palli D, Decarli A et al. (1990). A case–control study of gastric cancer and diet in Italy: II. Association with nutrients. International Journal of Cancer, **45**:896–901.

Bukowski J, Somers G, Bryanton J (2001): Agricultural contamination of groundwater as a posible risk factor for growth restriction and or prematurity. Journal of Occupational Medicine **43**:377-383

Burden R.J (1982). Nitrate contamination of New Zealand aquifers: A review. New Zealand Journal of Science, **25**: 205-220

Bureau of the Census (1993): The housing survey of the United States 1993. US Dept of Commerce, Economic and Statistics Administration Bureau of the Census.

Butler A; Williams D (1993): The physiological role of nitric oxide. Journal of Chemical Society Reviews **22**, 233- 241

Butler AR and Rider JH (2004): Formation of nitric oxide from nitrous acid in ischemic tissue and skin. Nitric oxide (2004) **10**: 20-24

CAEPA (1997): California Environmental Protection Agency: Public Health Goals for nitrate and nitrite in drinking water, California EPA 1997.

Calabrese, E. J., and Baldwin, L. A. (2001): Hormesis: A generalizable and unifying hypothesis. Critical Reviews & Toxicology **31**, 353–424.

Caldwell, K.L.; Makhmudov, A.; Ely, E.; Jones, R.L.; Wang, R.Y (2011): Iodine status of the U.S. population, National Health and Nutrition Examination Survey, 2005–2006 and 2007–2008. Thyroid, **21**, 419–427 (2011).

California Department of Health Services (1989): Guidelines for the assessment of carcinogenic substances. Sacramento, CA: CDHS.

Cancer Research UK (2016): 2014 Thyroid cancer incidence statistics: Published, June 2016. http://www.cancerresearchuk.org/cancer-info/cancerstats. Accessed 1/2/17

Carrasco N (2000): Thyroid iodide transport: The Na /I Symporter (NIS). In: The Thyroid: a fundamental and clinical text pp 52–61. (Braverman LE, Utiger RD, Eds. Werner & Ingbar, Philadelphia 8th edition: Lippincott, Williams & Wilkins.

Carlson, G. (1987): United States Environmental Protection Agency. Drinking Water Subcommittee Memorandum to Richard Griesemer: SAB Opinion on Nitrate/Nitrite. May 11, 1987.

Cantor KP, Lynch CF, Hildesheim ME, Dosemeci M, Lubin J, Alavanja M, Craun G (1998): Drinking water source and chlorination byproducts. I. Risk of bladder cancer. Epidemiology, **9**: 21-28.

Cantor KP (1997). Drinking water and cancer. Cancer Causes Control, **8**:292–308.
Capen C (1992): Pathophysiology of chemical injury of the thyroid gland. Toxicol Letters 64-65 Spec No:381-8

Capen C (1998): Correlation of mechanistic data and histopathology in the evaluation of selected toxic endpoints of the endocrine system. Toxicology Letters **102–103**:405–409.

Capen CC (1997): Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicologic Pathology **25(1)**:39–48.

Cao Y, Blount BC, Valentin-Blasini L, Bernbaum JC, Phillips TM, Rogan WJ (2010): Goitrogenic anions, thyroid-stimulating hormone, and thyroid hormone in infants. Environmental Health Perspective 2010; **118 (9):**1332–1337.

Carwile JL, Michels KB. (2011): Urinary bisphenol A and obesity: NHANES 2003–2006. Environmental Research **111(6):**825–830.

Cassens R.G (1997): Residual nitrite in cured meats. Food Technology **51**, 53-55.

Caulfield L, Richard S, Rivera J, Musgrove P, Black R (2006): Stunting, wasting, and micronutrient deficiency disorders 2006. In: Disease control in developing countries. Eds. Dean T, Jamison D T, Breman J, Measham R, Alleyne G.

Cavill N, Foster C, Oja P, Martin B W (2006): An evidence-based approach to physical activity promotion and policy development in Europe: Contrasting case studies. Promotion Education **13(2):**104–111.

CDC (1993a): Centre for Disease Control and Prevention. Public Health in the New American Health System. Discussion Paper. Atlanta, GA: Centres for Disease Control and Prevention, 1993.

CDC (1993b): Centre for Disease Control and Prevention Metropolitan Atlanta Congenital defects Programme Procedure Manual USDHHS, PHS Atlanta, GA, June 1993; pg201.

Chan WC, Fong YY (1977). Ascorbic acid prevents liver tumour production by aminopyrine and nitrite in the rat. International Journal of Cancer, **20**:268–270.

Chen AY, Jemal A, Ward EM (2009): Increasing incidence of differentiated thyroid cancer in the United States, 1988-2005. Cancer **115** (16):3801-7 (2009).

Chen C; Wang C (1990): Ecological correlation between arsenic level in well water and age adjusted mortality from malignant neoplasm. Cancer Research. **50**:5470-5474.

Chilton J (1996): Groundwater. In: Water quality Assessment. *Ed*. Chapman D. UNEP 413-510.

Chilvers C, Inskip H; Caygill C (1984): A survey of dietary nitrate in Well- water users. International Journal of Epedemiology; **13**:324 – 331 (1984).

Choi B (1985): N nitroso componds and human cancer: A molecular epidemiologic approach. American Journal of Epidemiology, **121**, 737(1985).

Chow CK, Chen CJ, Gairola C (1980): Effect of nitrate and nitrite in drinking water on rats. Toxicology Letters, **6**:199–206.

Churchill JE, Ashley DL, Kaye WE (2001): Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. Archieves of Environmental Health **56(2):**157–166.

Ciji A, Sahu N, Pal A, Akhtar M (2013): Nitrite-induced alterations in sex steroids and thyroid hormones of Labeo rohita juveniles: effects of dietary vitamin E and L-tryptophan, Fish Physiology & Biochemistry, **39** (2013) 1297–1307.

CIWEM (2004): Chattered Institute of Water and Environmental Management (CIWEM). Risk assessment for drinking water safety: what goes around comes around? Conference Introductory Notes.
http://www.ciwem.org/events/Risk_assessment_conference_141204.pdf. Accessed 11/11/13.

Coburn D, Poland B, (1996): The CAIR vision of the determinants of Health. A critique. Canadian Journal of Public Health, **87:** 308-310.

Colborn T, Clement C (1992): Chemically-induced alterations in sexual and functional development: the wildlife/human connection. In: Advances in Modern Environmental Toxicology (Mehlman MA, ed). Vol 21: Princeton, NJ: Princeton Scientific Publishing, 1992; 403.

Cole J, Brown C (1980): Nitrate reduction to ammonia by fermentative bactera: A short circuit in the biological nitrogen cycle. FEMs Microbiology Letters **7**, 65-72.

Comly H.H (1945): Cyanosis of infant caused by nitrates in well water. Journal of American Medical Association **129**, 112-116.

Comly (1987): Cyanosis in infants caused by nitrates in well water. Journal of American Medical Association 129: 112-116. Reprinted in 1987, Journal of American Medical Association **257 (20),** 2788-2792.

Conolly RB, Lutz W.K (2004): Non-monotonic dose-response relationships: mechanistic basis, kinetic modelling, and implications for risk assessment. Toxicological Science **77**:151–157.

Cordier S, Iglesias MJ, Le Goaster C, Guyot MM, Mandereau L, Hemon D (1994): Incidence and risk factors for childhood brain tumors in the Ile de France. International Journal of Cancer, **59**:776–782.

Cordier S, Bergeret A, Goujard J, Ha MC, Ayme S, Bianchi F, Calzolari E, De Walle HE, Knill-Jones R, Candela S, Dale I, Dananche B, de Vigan C, Fevotte J, Kiel G, Mandereau L (1997): Congenital malformations and maternal occupational exposure to glycol ether. Epidemiology **8**: 355-63.

Corvilain B, Van Sande J, Dumont J, Bourdoux P, Ermans A (1998): Autonomy in endemic goiter. Thyroid; **8:** 107-113 (1998).

Coss A, Cantor KP, Reif JS, Lynch CF, Ward MH (2004): Pancreatic cancer and drinking water and dietary sources of nitrate and nitrite. Americal Journal Epidemiology, **159**:693–701.

Council of the European Union (1991): Directive 91/271/EEC concerning urban waste water treatment. (OJ (1991) L271/40). (Urban Waste Water Directive).

Council of the European Union (1991). Directive 91/676/EEC (1991) concerning the protection of waters against pollution caused by nitrates from agricultural sources (OJ (1991) L375/1). Nitrates Directive.

Council of the European Union (1996): Directive 96/61/EC (1996) concerning integrated pollution prevention and control (IPPC Directive).

Council of the European Union (1998): Council Directive 98/83/EC on the quality of water intended for human consumption. (Drinking Water Directive) Official Journal of the European Communities; L330, pg 34, Nov 3 1998.

Council of the European Union (2006): Directive 2006/118/EC (2006) on the protection of groundwater against pollution and deterioration (OJ L 372, 27.12.2006, p.19).

Covello V, Merkhofer M (1993): Exposure assessment. In: Risk Assessment Methods: Approaches for Assessing and Environmental Risks. Platinum Press, New York, pg91-125.

Crain DA, Eriksen M, Iguchi T, Jobling S, Laufer H, LeBlanc GA, Guillette Jr LJ (2007): An ecological assessment of bisphenol-A: evidence from comparative biology. Reproductive. Toxicology **24**:225–239.

Crain DA, Janssen SJ, Edwards TM, Heindel J, Ho SM, Hunt P, Iguchi T, Juul A, McLachlan J, Schwartz J, Skakkebaek N, Soto A, Swan S, Walker C, Woodruff T, Woodruff T, Giudice L,

Guillette L (2008): Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. Fertility Sterility **90(4)**:911–940.

Craun G, Greathouse D, Gunderson D (1981): Methemoglobin level in young children consuming high nitrate well water in the US. International Journal Epidemiology **10**: 309-317.

Crews D, Willingham E, Skipper JK (2000): Endocrine disruptors: present issues, future directions. The Quaterly Review of Biology **75**:243–260.

Croen LA, Todoroff K, Shaw GM (2001): Maternal exposure to nitrate from drinking water and diet and risk of neural tube defects. American Journal of Epidemiology **153 (4)**: 325-31.

Crofton K, Craft E, Hedge J, Gennings C, Simmons J, Carhman R, Carter W, DeVito M (2005): Thyroid hormone disrupting chemicals: Evidence for dose-dependent additivity or synergism. Environmental Health Perspective **Vol.113**, No 11 pgs 1549-1554.

Crofton, K.M. (2008). Thyroid disrupting chemicals: mechanisms and mixtures. Int. J. Androl., 31(2): 209–223.

Croll BT, Hayes CR. (1988): Nitrate and water supplies in the United Kingdom. Environmental Pollution **50**:163–187.

Cross AJ, Pollock JR, Bingham SA (2003): Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Research, **63**:2358-2360 (2003).

Crump, K. S., Hoel, D. G., Langley, C. H., and Peto, R. (1976): Fundamental carcinogenic processes and their implications for low-dose risk assessment. Cancer Research. **36**, 2973–2979.

Crump K (1984): A new method for determining allowable daily intakes. Fundamental Applied Toxicology **4**, 854-871.

Crump K (1995): Calculation of benchmark dose from Continuous data. Risk Analysis **15**, 79-89.

Cuello C, Correa P, Haenzel W Haenszel W, Gordillo G, Brown C, Archer M, Tannenbaum S (1976): Gastric cancer in Colombia. I. Cancer risk and suspect environmental agents. Journal National Cancer Institute, **57**:1015–1020.

Cuello C, Lopez J, Correa P, Murray J, Zarama G, Gordillo G (1979): Histopathology of gastric dysplasias: correlations with gastric juice chemistry. American Journal of Surgical Pathology, **3**:491–500.

Culotta E, Koshland D.E (1992): No news is good news. Science 258, pp1862

Dagan R, Zaltzstein E, Gorodischer R (1988): Methaemoglobinemia in young infants with diarrhoea. European Journal of Pediatrics **147**:87–89.

Dahl, L.; Meltzer, H.M (2009): The Iodine Content of Foods and Diets: Norwegian Perspectives. In: Comprehensive Handbook of Iodine. Preedy, V.R., Burrow, G.N., Watson, R.R., Eds.; Academic Press: London, UK, 2009; pp. 345–352.

Danish E.H (1983): Methaemoglobinemia in infants with enteritis. Journal of Pediatrics **102**:162–163.

Daston GP, Cook JC, Kavlock RJ (2003): Uncertainties for endocrine disrupters: our view on progress. Toxicological Science **74(2**):245–252.

Davies D.B (2000): The nitrate issue in England and Wales. Soil use and management **16:** 142-144 (2000).

Davies JM (1980): Stomach cancer mortality in Worksop and other Nottinghamshire mining towns. British Journal of Cancer, **41**:438–445.

Davies L, Welch HG (2006): Increasing incidence of thyroid cancer in the United States, 1973-2002. Journal of American Medical Association; **295(18):** 2164-7 (2006).

Davis B & Klein A (1996): Medium- specific and multi medium risk assessment: In: Toxicology and risk Assessment: Principles, Methods and Applications, Pg271-291. (Eds) Fan A and Louis Chang L, (Mercel Dekker publishers 1996).

DeBenoist B, McLean E, Andersson M, Rogers L (2008): Iodine deficiency in 2007; global progress since 2003. Food Nutrition Bulletin; **29:** 195–202 (2008).

Deeks J.J; Higgins J.P.T; Altman D.G (editors) (2011): Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011.

DEFRA (2004): Department of Food and Rural Affairs): Department of Food and Rural Affairs. Nitrate Vulnerable Zones in England. http://www.defra.gov.uk/environment/water/quality/nitrate/nvz.htm. Accessed 12/2/13

DEFRA (2002): Contaminants in soil: Collation of toxicological data and intake values for humans. R&D Publication CLR 9. DEFRA/ EA 2002.

DEFRA & Envorinment Agency (2002): Assessment of risk to humans from land contamination: an overview of of the development of guideline value and related research. Report CLR 7.

De Groef B, Decallonne B, Van der Geyten S, Darras V, Bouillon R (2006): Perchlorate versus other environmental sodium/iodide symporter inhibitors: Potential thyroid –related health effects. European Journal of endocrinology **155**, pg 17-25.

De Kok TM, Engels LB, Moonen E, Kleinjans JC (2005): Endogenous formation of carcinogenic N-nitroso compounds in the colon of patients with chronic inflammatory bowel disease. Gut, **54**:731-732 (2005).

Delange, F (2002): Iodine deficiency in Europe and its consequences: An update. Eur. J. Nucl. Med. Mol. Imaging, **29**, S404–S416 (2002).

Delange F (2001): Iodine deficiency as a cause of brain damage. Postgraduate Medical Journal **77**, 217-220.

Delange F; deBenoist B; Burgi H; ICCIDD Working group (2002): Determining median urinary iodine concentration that indicates adequate iodine intake at population level. Bulletin of the World Health Organisation, **80 (8)**, 633-636 (2002).

Delange F, de Benoist B, Alnwick D (1999): Risk of iodine induced hyperthyroidism after correction iodine deficiency by iodised salt. Thyroid; **9**:545-546 (1999).

Delange F; Hetzel B.S (2003): The iodine deficiency disorders. IN: Thyroid Manager. Hennemann G, DeGroot L, eds. Endocrine Education, Inc., 2003. http://www.thyroidmanager.org/chapter20/chapter20.pdf. (Accessed 20/9/13).

Delange F (2001): Iodine deficiency as a cause of brain damage. Postgraduate Medical Journal, 2001, 77:217–220.

Delange F (1998): Screening for congenital hypothyroidism used as an indicator of the degree of iodine deficiency and of its control. Thyroid, **8**:1185–1192 (1998).

Delange F (1996): Administration of iodized oil during pregnancy: A summary of the published evidence. Bulletin of the World Health Organization, **74**:101–108 (1996).

DePaoli, K., Seal, J., Taylor, R (2012): Progressive improvements in iodine status in Tasmaniawith voluntary then mandatory fortification: preliminary results from the 2011Tasmanian Urinary Iodine Survey. Nutrution &.Dietetics **69**, 53.

De Roos A. J; Ward M. H, Lynch C F, Cantor K. P (2003): Nitrate in Public Water Supplies and the risk of colon and rectum cancer. Epidemiology 2003; **14**: 640 – 649.

Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC (2009): Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocrine Review **30 (4)**, 293–342

Dillion P.J, Ragusa S.R, Richardson S.B (1991): Biochemistry of a plume of nitrate-contaminated groundwater. In: Nitrate contamination: Exposure consequences and Control. Eds: Bogardi I and Kuzelka R. NATO ASI serial G: Ecological Sciences 309 Springer, Berlin, 173-180.

DOE (1994): Department of Environment (Welsh and Scottish Office). A manual on treatment of private water supplies. HMSO London.

DoH (2012): Department of Health: Statutory Guidance on Joint Strategic Needs Assessments and Joint Health and Wellbeing Strategies. www.dh.uk/publications. Accessed 25/10/2010

DoH (1991): Guildelines for the Evaluation of Chemicals for Carcinogenicity. Committee on carcinogenicity of chemicals in food, consumer products and environment. Report RHSS 42, HMSO London.

DoH (2010a): Department of Health. Healthy Lives, Health People: Our strategy for public health in England. London. (http://www.dh.gov.uk/en/publicationsandstatistics/publications/publicationspolicyandguidance/DH121941). Accesed 24/10/2014

DoH (2010b): Department of Health. Equity and Excellence: Liberating the NHS. London. (www.dh.gov.uk/en/healthcare/liberatingthenhs/index.htm). Accesed 24/10/2014

Dohan O, Carrasco N (2003): Advances in Na(+)/I(−) symporter (NIS) research in the thyroid and beyond. Mol.Cell Endocrinol. **213**:59–70 (2003).

Dohan O, De la Vieja A, Paroder V, et al. (2003): The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. Endocrine Review **24(1)**:48–77.

Dolby JM,Webster AD, Borriello SP, Barclay FE, Bartholomew BA, Hill MJ (1984): Bacterial colonisation and nitrite concentration in the achlohydric stomach of patients with primary hypogammaglobulinaemia or classic pernicious anaemia. Scandinavia Journal of Gastroenterology **19 (1)**, 105-110.

Doll R, Peto J (1985): Effects on human health of exposure to asbestos. Health and Safety Executive, HMSO, London.

Dorsch MM, Scragg RKR, McMichael AJ, Baghurst PA, Dyer KF (1984): Congenital malformation and maternal drinking water supply in rural South Australia: a case - control study. Journal of Epidemiology, 119: 473- 86 (1984).

Douglas M (1985): Risk Acceptability According to the Social Sciences. Russell Sage foundation New York 1985.

Dourson M, Felter S, Robinson D (1996): Evolution of Science-Based Uncertainty Factors in Non cancer Risk assessment. Regulatory Toxicology and Pharmacology 24, 108-120.

Doyle MP and Hoekstra JW (1981): Oxidation of nitrogen oxides by bound dioxygen in hemoprotein. Journal of Inorganic Biochemisty 14: 351-358 (1981)

Doyle MP, Pickering RA, Deweert TM, Hoekstra JW, Pater D (1981): Kinetics and mechanism of the oxidation of human deoxyhaemoglobin by nitrites. J Bio chem. (1981) **256**:12393-8.

Doyle TJ, Zheng W, Cerhan JR, Hong CP, Sellers TA, Kushi LH, Folsom AR. The association of drinking water source and chlorination by-products with cancer incidence among postmenopausal women in Iowa: a prospective cohort study. Am J Public Health (1997); **87**: 1168-1176.

Drapier J, Pellar C, Henry Y (1991): Generation of EPR- detectable nitrosyl-iron complexes in tumour target cell co-cultured with activated micropahges. J. Biol.Chem **266**, 10162-10167

Dremier S, Coppee F, Delange F, Vassart G, Dumont JE, Van Sande J (1996): Thyroid autonomy: Mechanism and clinical effects. Journal of Clinical Endocrinology and Metabolism, 1996, **81**:4187–4193.

Drinking Water Inspectorate (DWI) (2002): Chief Inspector's Report 2002.

Drozd V, Saenko V, Brenner A, Drozdovitch V, Pashkevich V; Kudelsky A, Demidchik Y, Branovan I, Shiglik N, Rogounovitch T, Yamashita S, Biko J, Reiners C (2015): Major factors affecting incidence of childhood thyroid cancer in Belarus after the Chernobyl accident: Do nitrates in drinking water play a role.
http://dx.doi.org/10.1371/journal.pone.0137226 p1-14

Druckrey H, Ivankovic S, Preussmann R (1966): Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea to pregnant rats. Nature, **210**:1378–1379.

Duijvenboden W. Van, Mathijsen A (1989): Intergrated criteria document Nitrate. Bilthoven. RIVM Report No. 758473012

Duncan C, Dougall H, Johnson P, Green S; Brogan R; Leifert C; Smith L; Michael Golden M; Benjamin N (1995): Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. Nature Medicine, **1,** 546-551

Duncan C, Li H, Dykhuizen R, Frazer R, Johnston P, MacKnight G, Smith L, Lamza K, McKenzie H, Batt L, Kelly D, Golden M, Benjamin N, Leifert C (1997): Protection against oral and gastrointestinal diseases. Importance of dietary nitratae intake, oral nitrate reduction and enterosalivary nitrate circulation. Comp. Biochem. Physiol.**118A**, 939-948.

Dunn, H.G., Robertson, A.M., and Crichton, J.V (1986): Clinical outcome: Neurologic sequelae and their evolution. In Sequelae of low birth weight: The Vancouver Study. Clinics in Developmental Medicine Series. H.G. Dunn, Ed. London: Mac Keith, 1986, pp. 68-96.

DWI (1996): Tap Water Consumption in England and Wales: Findings from the 1995 National Survey. Drinking Water Inspectorate, London. Report no. DWI0771.

DWI (2006): Drinking Water Inspectorate: Chief Inspectors Report, 2006.

DWI (2011): Drinking Water Inspectorate. Chief Inspector's Report 2011

DWI (2014): Drinking Water 2013: Private Water Supplies in England. A Report by the Chief Inspector of Drinking Water, July 2014.

DWI (2013): Drinking water 2012: Private Water Supplies in England. A Report by the Chief Inspector of Drinking Water, July 2013.

DWI (2010): Legislative background to the Private Water Supplies Regulations 2009. Section 9 (E&W) of the Private Water Supplies: Technical Manual 2010.

Dybing E, Doe J, Kleiner J, O'Brien J et al (2002). Hazard Chracterisation of chemicals in food and diet: Dose-response mechanism and extrapolation issues. Food Chem Toxicol. 40, 237-282.

Dykhuizen RS, Fraser A, Duncan C, Smith CC, Golden M, Benjamin B, Leifert C (1996): Antimicrobial effect of acidified nitrite on gut pathogens: importance of dietary nitrate in host defense. Antimicrobial Agents Chemotherapy 1996, **40**:1422-1425.

ECE (1993): Prevention and Control of water pollution from fertilisers and pesticides. Water series No 1. Protection of water resources and aquatic ecosystems pg. 35-50.

EHD (1981): Environmental Health Directorate. Tap Water Consumption in Canada. Health Protection Branch, Ottawa, Canada, Report 82-EHD-80.

Eichholzer M, Gutzwiller F (1998): Dietary nitrates, nitrites, and N-nitroso compounds and cancer risk: a review of the epidemiologic evidence. Nutr Rev, **56**:95–105.

ECETOC (1988): European Chemical Industry Ecology and Toxicology Centre (ECETOC): Nitrate and drinking water. Technical Report No 27.

EEDA (2009): East of England Development Agency. www.eeda.org.uk. Accessed 13/3/09

Eisenberg G, Spiegelhalder B, Preussmann R (1980): Carcinogenicity of N-nitroso-3-hydroxypyrrolidine and dose-response study with N-nitrosopiperidine in rats. IARC Scientific Publications, **31,** 657-666

Elder L, Knopp-Schnieder A. (1998): Statistical Models for low-dose exposure. Mutation **405**, 227-236.

Edino ST, Mohammed AZ, Ochicha O, Malami SA, Yakubu AA (2010): Thyroid cancers in nodular goiters in Kano, Nigeria. Nigeria J Clinical Practice. **13(3)**:298-300(2010)

Elrod JW, Calvert JW, Gundewar S, Bryan NS, Lefer DJ.Nitric oxide promotes distant organ protection: evidence for an endocrine role of nitric oxide. Proceedings of National Academy Science. 2008: **105 (32):**11430-5.

Ellenberg, J., and Nelson, K.B (1979): Birth weight and gestational age in children with cerebral palsy or seizure disorders. American Journal of Diseases of Children, **133**:1044-48.

El-Wakf A, Hassan H, Elsaid F, El-said A (2009): hypothyroidism in male rats of different ages exposed to nitrate polluted drinking water. Journal of Medicine and Medical science. Vol. **4**, issue 2, pp160-164

Ema M, Kanoh S (1998): Foetal toxicity of Potassium nitrate in second generation rats. Japanese Journalof Pharmacology **81 (6)** 469-80

Enhealth (2004): Environmental health risk assessment: Guidelines for assessing human health risks from environmental hazards: Department of Health and Ageing and enhealth council (Australia). http://enhealth.nphp.au. Assessed 9 Sept. 2011.

Enewold L, Zhu K, Ron E, Marrogi AJ, Stojadinovic A, Peoples GE, Devesa SS (2009): Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980-2005. Cancer Epidemiology Biomarkers & Prevention; **18(3)**:784-91.

Engle P. L, Black M, Behrman J, Cabral de Mello M, Gertler P, Kapiriri L; Reynaldo Martorell R; Young M (2007): International Child Development Steering Group (2007): International child development steering Group. Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world. Lancet 2007: 369 (9557): 229-42.

Environment Agency (2007a): Nitrate in drinking water - the current status in England (2006). ADAS report to DEFRA (2007).

Environment Agency (2007b): Environment Agency, UK. The unseen threat to water quality: Diffused water pollution in England and Wales report. May 2007 (http://www.environment-agency.gov.uk) Assessed 20/12/07.

Environment Agency (2002a): Environment agency, UK: The microbiology of drinking water (2002): Part 1 - Water quality and public health. Report by the Standing Committee of Analyst. www.dwi.gov.uk/regs/pdf/part1.pdf. Accessed 18/2/10

Environment Agency (2004): "Business as usual projections of agricultural outputs". Report by University of Cambridge.
http://www.environment-agency.gov.uk/commondata/103599/busasusual wfd_854912.doc. Accessed 18/2/10

Ericson A, Kallen B, Lofkvist E (1988): Environmental factors in the aetiology of neural tube defects: a negative study. Environmental Research, **45**:38–47.

Ershow, A. G., Brown, L. M. & Cantor, K. P. (1991): Intake of tap water and total water by pregnant and lactating women. American Journal of Public Health **81(3)**, 328–334.

Eskiocak S, Dundar C, Basoglu T, Altaner S (2005): The effects of taking chronic nitrate by drinking water on thyroid function and morphology. Clinical Experimental Medicine. **Vol. 5**, Issue 2, pp66-71.

Eskandari S, Loo D, Dai G, Levy O, Wright E, Carrasco N (1997): Thyroid Na+/I− symporter. Mechanism, stoichiometry, and specificity, Journal of Biological Chemistry, **272**, 27230 – 27238.

European Commision (1980): Directives on the quality of water for human consumption No 80/778. Off. Journal EEC 229: 11- 29 (1980)

European Commission (1994): Europe's Environment Statistical Copendium.

The Council of the European Union (1998): Council Directive 98/83/EC on the quality of water intended for human consumption. Official Journal of the European Communities; L330, pg 34, Nov 3 1998.

European Commission (2007): The quality of drinking water in the European Union, Synthesis Report on the Quality of Drinking Water in the European Union period 2002–2004. (Directives 80/778/EEC and 98/83/EC).

European Commission (2007) COM (2007) 120: Report from the Commission to the Council and the European Parliament on the implementation of the Council Directive 91/676/EEC concerning the protection of the waters against pollution caused by nitrates from agricultural sources for the period 2000–2003.

European Commission (2010) COM (2010) 47: Report from the Commission to the Council and the European Parliament on the implementation of the Council Directive 91/676/EEC concerning the protection of the waters against pollution caused by nitrates from agricultural sources based on Member States reports for the period 2004–2007.

European Commission (2002): Report from member states on implementing council nitrate directive 91/676/EEC. COM (2002) 407

European Council (1998): Council Directive on the quality of water intended for human consumption (98/83/EC). Official Journal of the European Communities, L330/32 EN (05.12.11).

European Commission: Agricultural and rural development. Appendix 2: EU milk consumption patterns, UK, France and Germany. http://ec.europa.eu/agriculture/eval/reports/schoolmilk/appendix2.pdf (accessed May 7, 2014).

European Environment Agency (EEA) (2004): European Environment Agency: Signals: A EEA update on selected issues.

Evrard P, Kadhim HJ, Saint-George P, Gadisseux J.F (1989): Abnormal development and destructive processes of human brain during second half of gestation. In: developmental neurobiology. Eds: P. Evrard et al. Raven press, NY.

EWG (1996): Environmental Working Group. Pouring it on: Health effects of nitrate exposure. www.ewg.org/reports/nitrate/pour_short.html. Accessed 12/12/14.

Ewing M, Mayon – White R (1951): Cyanosis in infancy from nitrates in drinking water. Lancet **260**, 931-934

Fagin D (2012): Toxicology: The learning curve. www.nature.com/news/toxicology-the-learning-curve-1.11644. Accessed 11/03/13.

Fan A.M, Steinberg VE (1996). Health implications of nitrate and nitrite drinking water: An update on methaemoglobinemia occurrence and reproductive and development toxicity. Regulatory Toxicology & Pharmcology **23** 35 – 43

Fan A.M, Willhite C.C, Brook S A (1987): Evaluation of the Nitrate drinking water standard with reference to infant methaemoglobinemia and potential reproductive toxicity. Regulatory Toxicology & Pharmacology. **7,** 135-148

Fan, A.M, Christensen J, Brown J (1992): Assessment of Nitrate Reproductive Toxicity. Presented at American College of Toxicity Meeting, San Francisco.

Fang FC (1997): Mechanisms of nitric oxide - related antimicrobial activity. Journal of Clinical Investigation **99:** 2818-25

Farland W, Dourson M (1992): Non-cancer health endpoints: approaches to quantitative Risk assessment. In: Comparative Environmental risk assessment. Ed: Cothern C. Lewis Publishers 1992

FDA (1972): Food and Drug Administration. Teratologic Evaluation of FDA 71-7 (Sodium Nitrate). Washington DC: US Department of Health and Human Services, Public health Services Publication 1972.

Feldt-Rasmussen U (2001): iodine and cancer. Thyroid 2001; **11(5):** 483-486.

Ferlay J, Shin HR, Bray F, et al. GLOBOCAN 2008 v1.2, Cancer incidence and mortality worldwide: IARC Cancer Base No. 10 . Lyon, France: International Agency for Research on Cancer; 2010. http://globocan.iarc.fr. Accessed March 5, 2014.

Fewtrell, L., and Kay, D (1996): Health risks from private water supplies. IN: NERC (2002). http://www.cieh.net/dload/pws/pdf/C10_pathogen_migration.pdf (accessed 19.03.11)

Fewtrell L, Kay D, Godfree A. (1998): The microbiological quality of private water supplies. Journal of Chartered Institute Water Environmental Management **12:**98–100.

Fewtrell L (2004): Drinking water nitrate, methaemoglobinemia and global burden of disease: A discussion. Environmental health perspective: Vol.112, **No. 14**; 1371-1374.

Fewtrell LJ, Prüss A, Landrigan P, Ayuso-Mateos JL (2004): Estimating the global burden of disease from environmental lead exposure. Environmental Research **94**(2) 120–133.

Finkel A (1990): Confronting uncertainty in risk management: A Guide for decision-makers. Washington DC.

Fischhoff B, Lichtenstein P, Slovic P, Kenney R, Derby S (1981): Acceptable risk. Cambridge University Press 1981.

Food Standards Agency (2000): A Report of the Study of Infectious Intestinal Disease in England. The Stationery Office: London.

Forman D, Al-Dabbagh S, Doll R (1985): Nitrates, nitrites and gastric cancer in Great Britain. Nature, **313**:620–625.

Forman D (2004): Nitrites, nitrates and nitrosation as causes of brain cancer in children: epidemiological challenges. International Journal Epidemiology, **33:**1216-1218

Franceschi S, Preston-Martin S, Dal Maso L, Negri E, La Vecchia C, Mack WJ, McTiernan A, Kolonel L, Mark SD, Mabuchi K, Jin F, Wingren G, Galanti R, Hallquist A, Glattre E, Lund E, Levi F, Linos D, Ron E. (1999): A pooled analysis of case-control studies of thyroid cancer. IV. Benign thyroid diseases. Cancer Causes Control **10**:583–595

Fraser P, Chilvers C (1981): Health aspects of Nitrates in drinking water. Science of the Total Environment **18,** 103 –116

Freedman DM, Cantor KP, Ward MH, Helzlsouer KJ (2000): A case–control study of nitrate in drinking water and non-Hodgkin's lymphoma in Minnesota. Archieves of Environmental Health, **55:**326–329.

Freedman NJ, Lefkowitz RJ (1996): Desensitization of G protein-coupled receptors. Recent Progress Hormone Research **51**: 319–351; discussion 352–353

Fried J.J (1991): Nitrates and their control in the EEC aquatic environment. In: In: Nitrate contamination: Exposure consequences and Control. Eds. Bogardi I and Kuzelka R. NATO ASI serial G: Ecological Sciences 309 Springer, Berlin, 173-180.

Freitag A, Thayer L, Leonetti C, Stapleton H, Hamlin H (2015): Effects of elevated nitrate on endocrine function in Atlantic salmon, *Salmo salar,* Aquaculture **436** (2015) 8–12.

Freitag A; Thayer L; Hamlin H (2016): Effects of elevated nitrate concentration on early thyroid morphology in Atlantic salmon (*Salmo salar Linnaeus*, 1758). Journal of Ichthyology: Volume 32, Issue 2; p. 296–301.

Frey, H.; Rosenlund, B.; Try, K.; Theodorsen, L (1993): Urinary excretion of iodine in Norway. In Iodine Deficiency in Europe; Delange, F., Ed.; Plenum Press: New York, NY, USA, 1993; pp. 297–300.

FSA (2008): Food Standards Agency. Retail survey of iodine in UK produced dairy foods. FSIS 02/08: 16 June 2008. http://www.food.gov.uk/multimedia/pdfs/fsis0208.pdf (accessed May 8, 2014).

FSANZ (2006): Food Standards Australia New Zealand. Cost benefits analysis of fortifying the food supply with iodine, 2006

Furtado, C, Adak, G.K., Stuart, J.M., Wall, P.G., Evans, H.S., and Casemore, D.P. (1998): Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992-5. Epidemiology and Infection (1998), **121**, 109-119.

Furukawa F, Nishikawa A, Ishiwata H, Takahashi M, Hayashi Y, Hirose M (2000): Renal carcinogenicity of concurrently administered fish meal and sodium nitrite in F344 rats. Japan Journal of Cancer Research, 91:139–147.

Galbraith, N.S., Barnett, N.J., and Stanwell-Smith, R (1987): Water and disease after Croydon: a review of waterborne and water associated disease in the UK 1937-86. Journal of the Institute of Water and Environmental Management, 1, 7-21

Gallego G, Goodall S, Eastman C.J (2010): Iodine deficiency in Australia: Is iodine supplementation for pregnant and lactating women warranted? Medical Journal of Australia, **192**: 461–63.

Gallagher M, Nuckols J, Stallones L, Savitz D (1998): Exposure to trihalomethanes and adverse pregnancy outcomes. Epidemiology, **9**: 484-489.

Gallo, J.E., and Lennerstrand, G (1991): A Population based study of ocular abnormalities in premature children aged 5 to 10 years: A regional study. American Journal of Ophthalmology (1991), **11**:539-47.

Gangolli S.D, Van den Brandt P.A, Feron V.J et al (1994): Nitrate, Nitrite and N-nitroso compounds. European Journal of Pharmacology **292:** 1-38

Garcia A.M, Fletcher T (1998): Maternal occupation in the leather industry and selected congenital malformations. Occupational & Environmental Medince; **55**: 284 – 6.

Garcia-Mayor RV; Rios M; Fluiters E; Mendez LF; Garcia-Mayor E.G; Andrade A(1999): Effect of iodine supplementation on a paediatric population with mild iodine deficiency. Thyroid, 1999, **9**:1089–1093.

Garthwaite J (1991): Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends in Neuroscience. **14**, 60-67

Gatseva P, Dimitrov I (1997): Population morbidity in a community with nitrate contamination of drinking water. Folia Medica, **39**:65–71.

Gatseva P, Vladeva S, Pavlov K (1998): Incidence of goitre among children in a village with nitrate contamination of drinking water. Folia Medica, **40**:19–23.

Gatseva P, Agriova M (2005): Iodine status of children living in area with high nitrate levels in drinking water. Archives of Environmental and Occupational Health, **Vol. 60, No. 6** pg 317-319.

Gatseva P, Agriova M (2008a): Iodine status and goitre prevalence in nitrate-exposed schoolchildren living in rural Bulgaria. Journal of the Royal Institute of Public Health **122**: 458-461.

Gatseva P, Agriova M (2008b): High – nitrate levels in drinking water may be a risk factor for thyroid dysfunction in children and pregnant women living in rural Bulgaria areas. International Journal of Hygiene and Environmental Health **211**, 555 – 559.

Gatseva P, Lazarova A, Maximova S, Pavlova K (1996): Experimental data on the effect of nitrates entering the organism with the drinking water, Folia Medica. (Plovdiv) **38** (1996) 75–83.

Gelperin A, Moses VK, Bridger C (1975): Relationship of high nitrate community water supply to infants and foetal mortality, Illinois Medical Journal **142 (2):** 155-157.

Geleperin A, Moses VJ, Fox G (1976). Nitrate in water supplies and cancer. Illinois Medical Journal, **149**:251–253.

Gerba C.P (1996): Risk assessment. In: Exposure Science. Eds: Pepper I; Gerba C & Brussseau M. Academic Press, San Diego, CA. 1996. Pgs. 345-364.

Ghasemi A & Zahedias S (2011): Is Nitric Oxide a Hormone? Iranian Biomedical Journal **15 (3**): 59-65 (July 2011)

Giles GG, McNeil JJ, Donnan G et al. (1994). Dietary factors and the risk of glioma in adults: results of a case–control study in Melbourne. Australia International Journal of Cancer, **59**:357–362.

Gilli G, Corroa G, Favilli S (1984): Concentration of nitrites in drinking water and incidence of gastric carcinomas. First descriptive study of the Piedmonte region, Italy. Science of the Total Environment **34**, 35-48.

Gitto E, Reiter RJ, Karbownik M, Tan DX, Gitto P, Barberi S, Barberi I (2002): Causes of oxidative stress in the pre- and perinatal period. Biology of Neonate 2002, **81**:146-157.

Glass G.V (1976): Primary, secondary and meta-analysis of research. Educational Researcher, 5: 3–8.

Gleeson C; Gray N (1997): The coliform index and water borne disease. Problems of microbial drinking water assessment. E&FN SPON Publishers.

Glinoer, D (2007): The importance of iodine nutrition during pregnancy. Public Health Nutrition 2007, **10**, 1542–1546.

GAO (1997): General Accounting Office, Washington DC. Information on the quality of water found at community water systems and private well GAO/RCED – 97-123.

GAO (1990): Drinking water compliance problems undermine EPA program as new challenges emerge, GAO/RCED-90-127, Washington, DC

Gochfeld M (2003): Why epidemiology of endocrine disruptors warrants the Precautionary principle. Pure Applied Chemistry Vol. 75, **Nos. 11-12**, pp. 2521-2529.

Gofti-Laroche, L., Potelon, J. L., da Silva, E. & Zrnirou, D. (2001): Description of drinking water intake in French communities (E.M.I.R.A study). Rev. Epidemiol. Sante Publique **49(5)**, 411–422.

Goldbolm RA, Tielemans E, Heedrerik D, Rubingh C et al (2006): Risk estimation for carcinogens on epidemiological data: a structured approach, illustrated by an example on chromium. Reg. Toxicol. and Pharm. Vol 44, Issue 3, pgs 294-310.

Goldman G.T, Mulholland J.A; Russell A.G, Strickland M.J, Klein M, Lance A, Waller L.A, Tolbert P.E (2011): Impact of exposure measurement error in air pollution epidemiology: effect of error type in time-series studies. Environmental Health **10**:61

Goodman MT, Hankin JH, Wilkens LR, Kolonel LN (1992): High-fat foods and the risk of lung cancer. Epidemiology, **3**:288–299.

Gonzalez CA, Riboli E, Badosa J, Batiste E, Cardona T, Pita S, Sanz JM, Torrent M, Agudo A (1994): Nutritional factors and gastric cancer in Spain. American Journal of Epidemiology, **139**:466–473.

Grau M, Subirana I, Elosua R, Fito M, Covas MI, Sala J, Masia R, Ramos R, Solanas P, Cordon F, Nieto FJ, Marrugat J; REGICOR Investigators (2010): Why should population attributable

fractions be periodically recalculated? An example from cardiovascular risk estimation in Southern Europe. Preventive Medicine 2010 Jul; **51(1**):78-84.

Gray R, Peto R, Brantom P, Grasso P (1991): Chronic nitrosamine ingestion in 1040 rodents: the effect of the choice of nitrosamine, the species studied, and the age of starting exposure. Cancer Research **51**. 6470-6491.

Green LC, Ruiz de Luzuriaga K, Wagner DA, Rand W, Istfan N, Young VR, Tannenbaum S.R.(1981): Nitrate biosynthesis in man. Proceedings of National Academy of Science, **78:** 7764-8.

Green, L.C., Tannenbaum, S.R. and Fox, J.G. (1982): Nitrate in human and canine milk. New England Journal of Medicine, **306(22)**: 1367–1368.

Greenblatt M, Lijinsky W (1972): Failure to induce tumors in Swiss mice after concurrent administration of amino acids and sodium nitrite. J National Cancer Institute, **48**:1389–1392.

Greenblatt M, Lijinsky W (1974): Carcinogenesis and chronic toxicity of nitrilotriacetic acid in Swiss mice. J. National Cancer Institute **52**:1123–1126.

Greenblatt M, Mirvish S, So BT (1971): Nitrosamine studies: induction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice. J. National Cancer Institute **46**:1029–1034.

Greenblatt M, Mirvish SS (1973): Dose–response studies with concurrent administration of piperazine and sodium nitrite to strain A mice. J National Cancer Institute **50**:119–124.

Greenland S, Robins JM (1988): Conceptual problems in the definition and interpretation of attributable fractions. American Journal of Epidemiology1988; **128**:1185-1197.

Greer M, Goodman G, Pleus R, Greer S (2002): Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. Environmental Health Perspectives, 110 (2002) 927–937.

Greer F & Shannon M (2005): Infant methaemoglobinemia: the role of dietary nitrate in food and water. Paediatrics (2005); **116(3);** 784-786.

Greg N.M (1941): Congenital cataract following German measles in the mother. Trans. Ophalmol Soc Australia **3**: 35-46

Grizzetti B, Bouraoui F, Billen G, Van Grinsven H, Cardoso A, Thieu V, Garnier J, Curtis C, Howarth R, Johnes P (2011): Nitrogen as a threat to European water quality. IN: The European Nitrogen Assessment, pg379-404. Eds: Mark A. Sutton, Clare M. Howard, Jan Willem Erisman, Gilles Billen, Albert Bleeker, Peringe Grennfelt, Hans van Grinsven and Bruna Grizzetti. Published by Cambridge University Press, 2011.

Groose Y; Baan R, Sraif K; Secretan B; El Ghissassai F; Cogliano V (2006): Carcinogenicity of nitrate, nitrite and cynobacterial peptide toxins. The Lancet Oncology. Vol. **7**, 628-9 (2006).

Gulis G, Czompolyova M, Cerhan JR (2002): An ecologic study of nitrate in municipal drinking water and cancer incidence in Trnava District, Slovakia. Environmental Research, **88**:182–187.

Gupta SK, Fitzgerald JF, Chong SK, Croffie JM, Garcia JG (1998): Expression of inducible nitric oxide synthase (iNOS) MRNA in inflamed oesophageal and colonic mucosa in paediatric population. American Journal Gastroentrology 1998 **93**, 795-798.

Gupta SK, Gupta RC, Gupta AB, Seth AK, Bassin JK, Gupta A.(2000): Recurrent acute respiratory infections in areas with high nitrate concentrations in drinking water. Environmental Health Perspective **108**:363–366.

Gupta SK, Gupta RC, Seth AK, Gupta AB, Bassin JK, Gupta A. (1999): Adaptation of cytochrome b5 reductase activity and methaemoglobinemia in areas with a high nitrate concentration in drinking-water. Bulletin WHO **77**:749–753.

Gupta, R. C., and Lutz, W. K. (1999): Background DNA damage from endogenous and unavoidable exogenous carcinogens: A basis for spontaneous cancer incidence? Mutat. Res. **424**, 1–8.

Grun F (2010): Obesogens. Curr Opin Endocrinol Diabetes. Obes **17**:453–459

Guzelian PS, Victoroff MS, Halmes NC, James RC, Guzelian CP (2005): Evidence-based toxicology: a comprehensive framework for causation. Human Exposure Toxicology 2005; **24**: 161-201.

Haidich AB (2010): Meta-analysis in medical research. Hippokratia, 14 (Suppl 1): 29-37

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ (1999): Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. New England Journal of Medicine, 1999; **341**:549–555.

Haldimann M, Alt A, Blanc A, Blondeau K (2005): Iodine content of food groups. Journal of Food Composition and Analysis; **8:** 171–217.

Hampel, R., Zollner, H., Glass, A. and Schonebeck, R. (2003): No influence of urinary nitrate excretion on the goitre prevalence in Germany. Med. Klin. (Munich), 98(10): 547–551.

Hanley N (1990): The economics of nitrate pollution. European Review Agricultural Economics, **17**:129-151

Hansen P. R, Taxvig C, Christiansen S, Axelstad M, Boberg J, Kiergaard M. K, Nellam C, Hass U (2009): Evaluation of endocrine disrupting effects of nitrates after in-utero exposure in rats and of nitrate and nitrite in the H295 and T – Screen Assay. Toxicology Science Vol. **108**, Issue **2**, pp437-444.

Hanukoglu A, Danon PN. (1996): Endogenous methaemoglobinemia associated with diarrheal disease in infancy. J Pediatric Gastroenterology & Nutrition **23**:1–7.

Haorah J, Zhou L, Wang X, Xu G, Mirvish SS. (2001): Determination of total N-nitroso compounds and their precursors in frankfurters, fresh meat, dried salted fish, sauces, tobacco, and tobacco smoke particulates. J Agric Food Chem **49**:6068–6078.

Haring, B. J. A., Karres, J. J. C., Poel, P. & van der Zoeteman, B. C. J. (1979): Research on the custom habits of drinking water consumption in the Netherlands. H_2O **12 (10),** 212–216.

Hartman PE (1983): Review: putative mutagens and carcinogens in foods. I. Nitrate/nitrite ingestion and gastric cancer mortality. Environ Mutag, **5**:111–121.

Harrison WN, Bradberry SM, Vale JA (2000). Chemical contamination of private drinking water supplies in the West Midlands, United Kingdom. J. Clinical Toxicology, **38**:137–144.

Hassan H, El-Wakf A, El-said F, El-Said A (2008): Hypothyroidism in male rats on different age exposed to nitrite polluted drinking water, Mansoura Journal of Forensic Medical Clinical Toxicology, **12**, 77–90.

Hatch EE, Nelson JW, Stahlhut RW, Webster TF (2010): Association of endocrine disruptors and obesity: perspectives from epidemiological studies. Int J Androl **33**:324–332.

Hawkes CH, Cavanagh JB, Darling JL *et al.* (1992): Chronic low-dose exposure of sodium nitrite in VM-strain mice: central nervous system changes. Hum Exp Toxicol, **11**:279–281.

Hawksworth G, Hill M.J (1974): The in vivo formation of nitrosamines in the rat bladder and their subsequent absorption. Br. J. Cancer 1974; **29**: 353-358.

Health Canada (1992): Background document for Canadian Drinking Water Guidelines: Nitrate/Nitrite, Ottawa.

Health Canada (1996): Chloramines, Ottawa. http://www.hc-sc.gc.ca/ewhsemt/alt_formats/hecs-sesc/pdf/pubs/water-eau/chloramines/chloramines-eng.pdf. Accessed 10/5/13

Health Canada (2006): Guidelines for Canadian Drinking Water Quality. Summary Table, Ottawa, Federal-Provincial-Territorial Committee on Drinking Water of the Federal-Provincial-Territorial Committee on Health and the Environment.

Health Canada (2013): Guidelines for Canadian Drinking Water Quality: Guideline Technical Document Nitrate and Nitrite (June 2013)

Hegesh E, Shiloah J (1982): Blood nitrates and infantile methemoglobinemia. Clinica Chemica Acta, **125**, 107-115

Heid IM, Küchenhoff H, Miles J, Kreienbrock L, Wichmann HE (2004): Two dimensions of measurement error: Classical and Berkson error in residential radon exposure assessment. Journal of Exposure Analysis and Environmental Epidemiology 14, 365–377.

Hennekens C, Buring J (1987): Epidemiology in Medicine. EDs.Mayrent S. Boston, Little, Brown & Co.

Herz-Picciotto I (1995): Epidemiology and quantitative risk assessment: A Bridge from Science to Policy. Am. Journal of Public Health. **Vol.85, No 4**, 484-491.

Hess, J; Millicent E; Tlumak J; Raab K; Luber G (2014): An Evidence-Based Public Health Approach to Climate Change Adaptation. Environmental Health Perspectives, Vol.122 no. 11. Pgs 1117-1186.

Hetzel BS (1994): The nature and magnitude of the iodine deficiency disorders. In: Hetzel BS, Pandav CS, eds. S.O.S for a billion. The conquest of iodine deficiency disorders. New Delhi, Oxford University Press, 1994: 3–26.

Hewitt A (1999): Structural birth defects. Developmental Biology; **216**, 423-425

Hiasa Y, Kitahori Y, Kitamura M, Nishioka H, Yane K, Fukumoto M, Ohshima M, Nakaoka S, Nishii S (1991): Relationships between serum thyroid stimulating hormone levels and development of thyroid tumours in rats treated with N-bis-(2-hydroxypropyl) nitrosamine. Carcinogenesis 1991, **12:**873-877

Hibbs C, Stencel E, Hill RM (1979): Nitrate toxicosis of cattle. Veterinary and human toxicology **21**: 401 – 403.

Hibbs J (1991): Synthesis of nitric oxide from L-arginine – a recently discovered pathway induced by cytokines with antitumor and antimicrobial activity. Res. Immunol. **142**-565-569

Hibbs JB, Vavrin Z, Taintor RR (1987): L-Arginine is required for expression of activated microphages effect or mechanism causing selective metabolic inhibition in target cells. J. immunology (1987) **138:** 550-65.

Higgins JPT, Thompson SG (2002): Quantifying heterogeneity in a meta-analysis. Statistics in Medicine; **21**: 1539-1558.

Higgins JPT, Thompson SG, Deeks J.J, Altman DG (2003): Measuring inconsistency in meta-analyses. Britich Medical Journal; 327: 557-560.

Higgy N.A, Verma A.K, Erturk E, Oberley T.D, El-Aaser A.A, El-Merzabani M.M, Bryan G.T.(1987): *Escherichia coli* infection of the Urinary Bladder: Induction of Tumours in Rats Receiving Nitrosamine Precursors and Augmentation of Bladder Carcinogenesis by *N*-Nitrosobutyl (4-hydroxybutyl)amine. IARC Scientific Pub. **No. 84**. Lyon: International Agency for Research on Cancer, 1987; 380-383.

Hill AB (1965): The environment and disease: Association or causation? Proceedings of the Royal Soceity of Medicine **58**:295-3000.

Hill MJ, Hawksworth G, Tattersall G (1973): Bacteria, nitrosamines and cancer of the stomach. Br.J Cancer, **28**:562–567.

Hillel S.I, Nachman G. (1972): Epidemiological and toxicological aspects of Nitrates and Nitrites in the Environment. Am. J Public Health. **Vol. 62, No 8**, 1045-1052.

Hirose M, Fukushima S, Hasegawa R, Kato T, Tanaka H, Ito N (1990): Effects of sodium nitrite and catechol or 3- methoxycatechol in combination on rat stomach epithelium. Japan Journal of Cancer Research, **81**:857–861.

Hirose M, Tanaka H, Takahashi S, Futakuchi M, Fukushima S, Ito N (1993): Effects of sodium nitrite and catechol, 3- methoxycatechol, or butylated hydroxyanisole in combination in a rat multi-organ carcinogenesis model. Cancer Research, **53**:32–37.

Hjelt K, Lund JT, Scherling B, Bendixen S, Lundstrom K, Stovring S, Voldsgarrd P, Linnet K (1995): Methaemoglobinaemia among neonates in a neonatal intensive care unit. Acta Pediatr **84(4):**365–370. 1995.

Hoek G; Mennen MG; Allen G; Hofschreuder P; Van Der Meulen T (1996): Concentration of acidic air pollutants in the Netherlands. Atmospheric Environment **30**, 3141-315

Hoering H, Chapman D, eds. (2004): Nitrate and Nitrite in Drinking Water: WHO Drinking Water Series. London: IWA Publishing.

Hogg N (2000): Biological chemistry and clinical of S-nitrosothiols. Free Radic Biol Med (2000) **28:** 1478-86

Hollowell JG, Hannon WH (1997): Teratogen update: Iodine deficiency, a community teratogen. Teratology, 1997, **55**:389–405.

Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE: (2002): Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). The Journal of clinical endocrinology and metabolism 2002, **87**:489-499.

Holt M.S (2000): Sources of chemical contaminants and routes into freshwater environment. Food and Chemical Toxicology **38,** 21-27.

Hopkin, S. M. & Ellis, J. C. (1980): Drinking Water Consumption in Great-Britiain. A Survey of Drinking Habits with Special Reference to Tap-water Based Beverages. Technical report TR137. Water Research Centre, Medmenham.

Höring H, Nagel M, Haerting J (1991). The nitrate-dependent endemic thyroid areas. P.147-53 In: Uberla K, Rienhoff U, Victor N (Eds.) Quantitative methods in the epidemiology. Berlin: Guugenmoos-Holzmann; 1991

Horing H, Dobberkau H, Seffner W (1988): Environmental anti-thyroid chemicals,
Z. Gesamte Hyg. 34, 170–173.

Hotchkiss J (1988): In: Food toxicology, a perspective on the relative risk. Eds. S.L Taylor, Scandan 57-100

Horton S (2006): The economics of food fortification: The Journal of Nutrition **(136):** 1068-1071.

Hrudley S, Krewski D (1995): Is there a safe level of exposure to a carcinogen? Environmental Science Technology. **29**:390A-375

Hsai C (1998): Respiratory function of haemoglobin. New England Journal of Medicine **338**, 239-247.

HSE (1996): Health Safety Executive. EH64 Summary Criteria for Occupational Exposure Limits. HSE books, Sudbury.

Hsin –Wei Kuo; Trong – Neng Wu; Chung –Yuh Yang (2007): Nitrates in drinking water and risk of death from rectal cancer in Taiwan. J. Toxicology & Env. Health **Part A, 70**: 1717-1722.

Hudak P (2000): Regional trends in Nitrate content of Texas groundwater. Journal of Hydrology **228**, 37-47.

Hudak PF (2003): Occurrence of nitrate and arsenic in alluvium and bolson aquifers of west Texas, USA. Bulletin of Environmental Contamination & Toxicology, **71**:905–911.

Hudak P.F, Sanmanee S (2003): Spatial patterns of nitrate, chloride, sulfate, and fluoride concentrations in the Woodbine Aquifer of north-central Texas. Environ Monit Assess, 82:311–320.

Hudak PF, Wachal D.J (2001): Oil production, agriculture, and groundwater quality in the southeastern Gulf Coast Aquifer, Texas. Environmental Monitoring & Assessment, **72:** 249 – 264.

Hunault C, Lambers A, Mensinga T, Van Isselt J, Koppeschaar H, Meulenbelt J (2007): Effects of sub-chronic nitrate exposure on the thyroidal function in humans, Toxicology Letters. **175** (2007) 64–70.

Hunt, D, Dee A, Oakes D (2004): Updating the estimates of the source apportionment of N to UK waters. Phase 2. DEFRA Report by WRc plc. http://www.fwr.org/defrawqd/wqd0002.htm. Accessed 1/11/12.

Hunter, P. R., Hughes, S., Woodhouse, S., Syed, Q., Verlander, N. Q., Chalmers, R. M., Morgan, K., Nichols, G., Beeching, N. & Osborn, K. (2004): Sporadic cryptosporidiosis case-control study with genotyping. Emerging Infectious Diseases, **10** (7)1241-9. http://www.cdc.gov/ncidod/EID/vol10no7/03-0582.htm. Accessed 10/11/13.

Hynes KL, Otahal P, Hay I & Burgess JR (2013): Mild iodine deficiency during pregnancy is associated with reduced educational outcomes in the offspring: 9-year follow-up of the gestational iodine cohort. Journal of Clinical Endocrinology and Metabolism 2013 **98** 1954–1962.

IARC (1977): International Agency for Research on Cancer (IARC). Monographs programme on the evaluation of the carcinogenic risk of chemicals to humans. Preamble (IARC intern. tech. report No 77/002).

IARC (1978): International Agency for Research on Cancer (IARC). Monographs on the evaluation of carcinogenic risks of chemicals to man. Vol 17: Some N-nitroso compounds. WHO 1978

IARC (1982): International Agency for Research on Cancer. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Supplment 4. Lyon France.

IARC (1983): International Agency for Research on Cancer (IARC). Approaches to classifying chemical carcinogens according to mechanism of action (IARC intern. tech. report No 83/001).

IARC (2010): International Agency for Research on Cancer. Ingested nitrate and nitrite, and cynobacterial peptide toxins. IARC Monograph (Vol.94) on the evaluation of carcinogenic risk to humans: International Agency for Research on Cancer 2010.

ICAIR (1987): Drinking water Criteria document on Nitrate/nitrite. USEPA office of drinking water, Washington DC.

Ignarro LJ, Buga GM, Wood KS et al (1987): Endothelium-derived relaxing factor produced and released form artery and vein is nitric oxide. Proc Natl Acad Sci USA (1987) **84**: 9265-9

Ignarro L (1989): Heme- dependent activation of soluble guannylate cyclise by nitric oxide-regulation of enzyme activity by porphyrins and metalloporphyrins. Semin. Haematology **26**, 63-76.

Ilnitsky AP, Kolpakova AS (1997): The enhancing effect of sodium nitrite on virus-induced leukemia in mice. Cancer Detection & Prevention, **21**:312–318.

Inui N, Nishi Y, Taketomi M, Mori M (1979): transplacental action of sodium nitrite on embrayonic cells of syrian golden hamster. Mutat. Res **66:** 149-158

IOM (1988): Institute of Medicine. Committee for the Study of the Future of Public Health.The Future of Public Health. Washington,D.C: National Academy Press, 1988

IPCS (2002): International Programme on Chemical Safety: Global Assessment of the State-of-the-Science of Endocrine Disruptors. WHO/PCS/EDC/02.2 (Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G, eds). World Health Organization, Geneva.

IPCS/WHO (2004): International Programme on Chemical Safety: Risk Assessment Terminology Part I & II. Harmonisation Project Document No. 1. World Health Organization, Geneva (2004).

IRR (2005a): Risk Estimation. Institute of Risk Research. Risk Management Framework: Step 3 Network for Environmental Risk Assessment and Management. University of Waterloo, Canada 2005.

IRR (2005b): Dealing with Uncertainty. Institute of Risk Research. Fundamental concepts in Risk Management: Network for Environmental Risk Assessment and Management. University of Waterloo, Canada 2005.

Isacson P. (1988): Proceedings of Technical Workgroup, Agricultural, Occupational and Environmental Health: Policy Strategy for the Future. Iowa City: Department of Preventive Medicine and Environmental Health. The University of Iowa, 1988; 18-21.

Ivankovic S (1979): Teratogenes and carcinogenic effects of chemicals during prenatal life in rats, syrian hamsters and minipigs. National cancer institute monograph 1979, **51:** 103-115

Jack G, Sharma V (1983): Nitrogen Circulation and nitrate in groundwater in an agricultural catchment in southern India. Environmental Geology **5(2)** 61-64

Jaekle RK, Lutz PD, Rosenn B, Siddiqi TA, Myatt L (1994): Nitric Oxide metabolites and preterm pregnancy complications. American-Journal of Obst and Gynaecology **171(4)**: 111-119

Jaffe ER (1981): Methemoglobinemia. Clinical Haematology, **10,** 99-122

Jahreis G, Hesse V, Schone F, Hennig A, Gruhn K (1986): Effect of chronic dietary nitrate and different iodine supply on porcine thyroid function, somatomedin-C-level and growth. Exp Clinical Endocrinology; **88(2):** 242–248.

Jahreis G, Hesse V, Rohde W, Prange H, Zwacka G (1991): Nitrate-induced hypothyroidism is associated with a reduced concentration of growth hormone releasing factor in hypothalamic tissue of rats, Exp. Clinical Endocrinology, **97;** 109–112.

Jan M.S, Van M, Welle I. J, Hageman G, Dallinga JW, Mertens PL, Kleinjans JC (1996): Nitrate contamination of drinking water: Relationship with HPRT variant frequency in Lymphocyte DNA and urinary excretion of N-Nitrosamines. Environmental Health Perspetive Vol. **104**, No 5, 522-8.

Janssen L, Visser H, Roemer F (1989): Analysis of large scale sulphate, nitrate, choloride and ammonia concentration in the Netherlands using an aerosol measuring network. Atmospheric Environment, **23(12):** 2783-2796).

Jensen OM (1982): Nitrate in drinking water and cancer in northern Jutland, Denmark, with special reference to stomach cancer. Ecotoxicology & Environmental Safety, **6**:258–267.

Johnson J.L et al (1983): Nitrate exposure in prenatal beef calves. Aver. Assn. Veterinary Laboratory diagnosticians 26th annual proceedings.

Johnson JL, Grotelueschen D, Knott M (1994): Evaluation of Bovine perinatal nitrate accumulation in Western Nebraska. Veterinary and Human Toxicology **36(5)**: 467-71

Jones B. H; Canham-Chervak M; Sleet D. A. (2010): Evidence - based public health approach to injury priorities and prevention recommendations for the U.S. military. American Journal of Preventive Medicine **38** (1 suppl.):s1–s10.

Jonklaas J, Nsouli-Maktabi H, Soldin S.J. (2008): Endogenous thyrotropin and triiodothyronine concentrations in individuals with thyroid cancer. Thyroid 18:943–952

Juhasz L, Hill MJ, Nagy G (1980): Possible relationship between nitrate in drinking water and incidence of stomach cancer. IARC Sci Publ, **97**:619–623.

Jurek AM, Greenland S, Maldonado G, Church TR(2005): Proper interpretation of non-differential misclassification effects: expectations vs observations. International Journal of epidemiology **34**:680-687

Kalkhoff S; Barnes K; Becher K; Savoca M; Schnoebelen D; Sadorf E; Porter S, Sullivan D (2000): Water Quality in the Eastern Iowa Basins 1991-98. U.S Geological Survey 1210:1-37

Kalter H, Warkany (1983): Congenital Malformations: Etiologic Factors and their Role in Prevention. The New England J Med **308**: 424 - 431, 491 – 6.

Kamiyama S, Ohshima H, Shimada A, Saito N, Bourgade MC, Ziegler P, Bartsch H (1987): Urinary excretion of N-nitrosamino acids and nitrate by inhabitants in high- and low-risk areas for stomach cancer in northern Japan. IARC Sci Publ, **84**:497–502.

Kaplan S, Novikov I, Modan B (1997): Nutritional factors in the etiology of brain tumors: potential role of nitrosamines, fat, and cholesterol. Am J Epidemiology, **146**:832–841.

Kaur S; Nieuwenhuijsen M; Ferrier H; Steer P (2004): Exposure of pregnant women to tap water related activities. Occup Environ Med. 2004 May; **61(5)**: 454–460.

Kay MA, O'Brien WO, Kessler B, McVie R, McCabe ERB. (1990): Transient organic aciduria and methemoglobinemia with acute gastroenteritis. Pediatrics **85**:589–592

Kovalchik S (2013): Tutorial on meta-analysis in R: R user's conference 2013. www.file//users/kovalchiks/master/tutorial/users2013/index.html#200. Accessed 1/12/16.

Laurberg P, Nohr SB, Pedersen KM, Hreidarsson AB, Andersen S, Bulow Pedersen I, Knudsen N, Perrild H, Jorgensen T, Ovesen L (2000): Thyroid disorders in mild iodine deficiency. Thyroid, 2000, **10**:951–963.

Levin HM, Jamison D, Breman J (1993): Micronutrient deficiency disorders. In: Disease control priorities in developing countries. New York, Oxford University Press, 1993: 421–451. Editors**:**Dean T. Jamison, Joel G. Breman, Anthony R. Measham, George Alleyne, Mariam Claeson, David B. Evans, Prabhat Jha, Anne Mills and Philip Musgrove

Lijinsky W (1986): The significance of N-nitroso compounds as environmental carcinogens. J Environ Sci Health 1986, **C4**:1-45.

Lundberg, J.O. & Govoni, M. (2004): Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Radic. Biol. Med. **37**: 395-400.

Kahaly G.J & Dietlein M (2002): Cost estimationof thyroid disorders in Germany. Thyroid **12** 909–914.

Kanno J, Matsuoka C, Furuta K, Onodera H, Miyajima H, Maekawa A, Hayashi Y (1990). Tumor promoting effect of goitrogens on the rat thyroid. Toxicologic Pathology 1990; 18 (2):239–46.

Keating JP, Lell ME, Strauss AW, Zarkowsky H, Smith GE. (1973): Infantile methemoglobinemia caused by carrot juice. New England J. Medicine **288(16):**824–826.

Kelley S, Oehme F, Hoffman S (1974): Effect of chronic dietary nitrates on canine

thyroid function, Toxicol. Appl. Pharmacol. 27, 200–203.

Kettner, P.M., Moroney, R.M., Martin, L.L. (2008): Designing and managing programs: An effectiveness-based approach. Los Angeles, Sage.

Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, Prins GS (2007): An evaluation of evidence for the carcinogenic activity of bisphenol A. Reprod Toxicol **24**:240–252.

Khoury MJ, Stewart W, Weinstein A, Panny S, Linsay P, Eisenberg M (1988): Residential mobility during pregnancy: implications for environmental teratogenesis. J Clinical Epidemiology **41**:15–20. 1988.

Kibiridge MS, Hutchison S, Owen CJ, Delves HT (2004): Prevalence of maternal dietary iodine insufficiency in the northeast of England: implications for the foetus. Arch Dis ChildFetal Neonatal Ed **89**:F436–F439

Kilfoy B, Zhang Y, park Y, Holford T, Schatzkin A, Hollenbeck A, Ward M (2011): Dietary nitrate and nitrite and risk of thyroid cancer in the NIH-AARP diet and health study. International Journal of Cancer **129**: 160-172.

Kilfoy B**,** Heltshe S**,** Nuckols J**,** Sabra M**,** Alan R Shuldiner A**,** Mitchell B**,** Matt Airola M**,** Holford T**,** Zhang Y**,** Ward M (2012): Modelled nitrate levels in well water supplies and prevalence of abnormal thyroid conditions among the Old Older Amish in Pennsylvania. Environ Health 2012, **11**:6. http://www.enjournal.net/content/11/1/6 accessed April 5 2012.

Kimmel C.A, Francis E.Z (1990a): Proceedings of the workshop on the acceptability an interpretation of dermal developmental toxicity studies. Fundamental Applied Toxicology **14**: 386-398.

Kimmel C.A, Rees DC, Francis E.Z (1990b): Proceedings of the workshop on the qualitative and quantitative comparability of human and animal development neurotoxicity. Nerotoxicology and Teratology **12**; 173-272.

Kirk S, Seymour K, Fletcher S, Davies W, Cheney C S and Adams B (2000): Small Licence-exempt Groundwater Sources. BGS Technical Report WD/00/15/EA NGCLC Project Report NC/06/06. BGS Keyworth.

Kirkwood B; Sterne J (2003): Measurement error: Assessment and Implications. In: Essential Medical Statistics. Second Edition, Blackwell Science 2003 pg429

Knekt P, Juvinen R, Dich J, Hakulinen T (1999): Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. International Journal of Cancer, **80**:852–856.

Knight TM, Forman D, Pirastu R, Comba P, Iannarilli R, Cocco PL, Angotzi G, Ninu E, Schierano S (1990): Nitrate and nitrite exposure in Italian populations with different gastric cancer rates. International Journal of Epidemiology, **19**:510–515.

Kohn MC, Melnick RL (2002): Biochemical origins of the non-monotonic receptor-mediated dose-response. Journal of Molecular Endocrinology **29**:113–123.

Knight T, Pirastu R, Palli D, Cocco P, Leach S, Packer P, Iannarilli R, Manca P, Moller H, Forman D (1992): Nitrate and N-nitrosoproline excretion in two Italian regions with contrasting rates of gastric cancer: the role of nitrate and other factors in endogenous nitrosation. International Journal of Cancer, **50**:736–739.

Knobeloch L, Krenz K, Anderson H (1992): Methaemoglobinemia in an infant-Wisconsin. Morbidity Mortality Weekly Report (MMWR) **42**:217. 1993.

Knobeloch L, Salna B, Hogan A, Postle J, Anderson H (2000): Blue Babies and Nitrate contaminated well water. Environmental Health Perspective**108 (7)** 675-678

Knobeloch L, Anderson H (2001): Causes of methaemoglobinemia: illness versus nitrate exposure. Environmental Health Perspective **Vol 109(1)** pgsA12-A14

Knox E.G (1972): Anencephalus and dietary intake. Br J. Pre. Soc. Med **26,** 219-223

Knowles, R.G. and Moncada, S. (1994): Nitric oxide synthases in mammals. Biochem. J. **298** (Part 2): 249-258.

Kochukov MY, Jeng YJ, Watson CS (2009): Alkylphenol xenoestrogens with varying carbon chain lengths differentially and potently activate signaling and functional responses in GH3/B6/F10 somatomammotropes. Environmental Health Perspective **117**:723–730.

Koplin D, Burkart M, Thurman E (1994): Herbicides and nitrates in near surface aquifers in the Mid Continental United States. US Geological Water-Supply Paper **2413**, 1-34

Korenbrot, C., Clayson, Z., Gill, A., and Patterson, E (1993): Evaluation of the implementation of the Comprehensive Perinatal Service Program. Final Report. San Francisco: Institute for Health Policy Studies, University of California, 1993.

Kohn M.C, Melnick R.L (2002): Biochemical origins of the non-monotonic receptor-mediated dose-response. J Molecular Endocrinology **29**:113–123.

Kostogrys R B, Pisulewski P W, Pecio A (2006): Nitrate affects thyroid status and serum tricyglycerols in Wister rats. Polish Journal of Food and Nutrition Science. **Vol.15/56, No.1:** pp71-76.

Kozianowski, G., Roberfroid, M. and Schi1ter, B. (1999): The application of in- vitro data in the derivation of the acceptable daily intake of food additives. Food and Chemical Toxicology, **37,** 1175-1197.

Krafte-Jacobs B, Brilli R, Szabo C, Denenberg A, Moore L, Salzman AL (1997): Circulating methemoglobin and nitrite/nitrate concentrations as indicators of nitric oxide overproduction in critically ill children with septic shock. Critical Care Medicine **25(9):**1588–1593. 1997.

Kramer M.S. Determinants of low birth weight (1987): Methodological assessment and meta-analysis. Bulletin of the World Health Organization (1987); **65**:663-737.

Kramer HJ, Jansen E, Zeilmaker MJ, Van Kranen HJ, Kroese ED (1995): Quantitative methods in toxicology for human dose-response assessment. An Overview. National Inst. of Public Health and Env. Protection, Bilthoven. Report No. 659101004

Kraybill, E.N., Bose, C.L., and D'Ercole, A.J (1987): Chronic lung disease in infants with very low birth weight. American Journal of Diseases of Children (1987) **141**:784-88.

Kross B.C, Hallberg G.R, Bruner D.R, Cherryholmes K, Johnson J.K (1993): The Nitrate contamination of Private Well water in Iowa. Am. J Public Health 1993; **83** 270- 272.

Kross C.K, Ayebo AD, Fuortes L.J (1992): Methemoglobinemia: Nitrate toxicity in Rural America. American Family Physician **46**, 183-188

Kumar R, Tandon R, BadamiK (1989): Co-existing hereditary methemoglobinemia and heterozygous B -thalassemia. Acta Paediatr. Scand. **78**,149-151

Kundi M (2006): Causality and the interpretation of epidemiologic evidence. Environmental Health Perspective 2006; **114**: 969-74.

Kuper F, Til H (1995): Subchronic toxicity experiments with potassium nitrite in rats. In: Health aspects of nitrates and its metabolites (particularly nitrites): Proceedings of an international workshop. Bilthoven (Netherlands), 8-10 November 1994, Council of Europe Press, Strasbourg. Pg 195-212.

Krewski D, Ryzin V (1981): Dose response models for quantal response toxicity data. In: Statistics and related topics, pp 201-231: Eds: M Csorgo, D Dawson, Roa J, Saleh E) North – Holland Amsterderm.

Krewski, D., Gaylor, D. W., and Lutz, W. K. (1995): Additivity to background and linear extrapolation. In Low-Dose Extrapolation of Cancer Risks: Issues and Perspectives (S. Olin, W. Farland, C. Park, L. Rhomberg, R. Scheuplein,T. Starr, and J. Wilson, Eds.), pp. 105–121. ILSI/International Life Sciences Institute, Washington, DC.

Langer P (2008): Possible effects of environmental nitrates and toxic organocholories on human thyroid in highly polluted areas in Slovakia. Thyroid: **Vol.18**, **No 3**, pg 353-362.

Lagerstedt, E., Jacks, G. and Sefe, F. (1994): Nitrate in groundwater and N circulation in eastern Botswana. Environmental Geology **23**: 60-64.

Laven RA, Biggadike HJ, Allison RD (2002): The effect of pasture nitrate concentration and concentrate intake after turnout on embryo growth and viability in lactating dairy cow. Reprod. Domest. Animal 2002 **37(2):** 111-115

Lazarus J H, Smyth P.P (2008): Iodine deficiency in the UK and Ireland. Lancet 2008; **372**: 888.

Leach SA, Thompson M, Hill M (1987): Bacterially catalysed N-nitrosation reactions and their relative importance in the human stomach. Carcinogenesis, **8**:1907–1912.

Leaf, C.D., Wishnok, J.S. and Tannenbaum, S.R. (1989): L-arginine is a precursor for nitrate biosynthesis in humans: Biochemical and Biophysical Research Communications, **163,** 1032-1037.

Lebby T, Roco JJ, Arcinue EL. (1993). Infantile methaemoglobinemia associated with acute diarrheal illness. American Journal Emergency Medicine **11**:471–472.

Lee C, Weiss R, Horvath D.J. (1970): Effects of nitrogen fertilisation on the thyroid function of rats fed 40 per cent orchard grass diets. Journal of Nutrition **100**: 1121-1126.

Lee, K., Greger, J.L., Consaul, J.R., Graham, K.L. and Chinn, B.L. (1986): Nitrate, nitrite balance and de-novo synthesis of nitrate in humans consuming cured meat. American Journal of Clinical Nutrition, **44**, 188-194.

L'hirondel J, L'hirondel J-L. (2002): Nitrate and man: toxic, harmless, or beneficial? Wallingford, Oxfordshire, UK: CABI Publishing.

Lesser B, Bruchovsky N (1974): Effect of duration of the period after castration on the response of the rat ventral prostate to androgens. Biochem J **142**:429–431

Leux C, Guénel P (2010): Risk factors of thyroid tumors: role of environmental and occupational exposures to chemical pollutants. Rev Epidemiol Sante Publique; **58(5):**359-67. doi: 10.1016/j.respe.2010.05.005. Epub 2010 Oct 25.

Levallois P, Ayotte P, Louchini R, Desrosiers T, baribeau H, Phaneuf D, Gringras S, Dumas P, Zee J, Poirier G (2000a). Sources of nitrate exposure in residents of rural areas in Quebec, Canada. Journal of Exposure Analysis & Environmental Epidemiology, **10**:188–195.

Levallois P, Ayotte P, Van Maanen JM, Desrosiers T, Gingras S, Dallinga JW, Vermeer IT, Zee J, Poirier G (2000b): Excretion of volatile nitrosamines in a rural population in relation to food and drinking water consumption. Food Chemistry & Toxicology **38**:1013–1019.

Levallois P, Theriault M, Rouffignat J Rouffignat J, Tessier S, Landry R, Ayotte P, Girard M, Gingras S, Gauvin D, Chiasson C (1998). Groundwater contamination by nitrates associated with intensive potato culture in Qubec. Science of the Total Environment, **217**:91–101.

Levandowski B A, Sharma P, Lane S.D, Webster N, Nestor A M, Cibula D A, Huntington S (2006): Parental literacy and infant health: An evidence-based healthy start intervention. Health Promotion Practice, **Vol.7 (1):**95–102.

Levin M.L (1953): The occurrence of lung cancer in man (1953): Acta Union International Contra Cancrum.**9**:531-541.

Levine JJ, Pettei MJ, Valderrama E, Gold DM, Kessler BH, Tractman H. (1998): Nitric oxide and inflammatory bowel disease: evidence for local intestinal production in children with active colonic disease. Journal of Pediatric Gastroenterology & Nutrition **26**(1):34–38.

Levy J (2009): An overview of science and decisions: Advancing risk assessment. Risk in Perspective Vol. 17, **Issue 1.** Havard Centre for Risk Analysis (2009).

Li M, Waite KV, Ma G, Eastman CJ (2006): Declining iodine content of milk and re-emergence of iodine deficiency in Australia. Medical Journal of Australia, **184**:307.

Li, M.; Eastman, C.J (2012): The changing epidemiology of iodine deficiency. Nature Reviews. Endocrinology, **8,** 434–440.

Lijinsky W (1984): Induction of tumours in rats by feeding nitrosatable amines together with sodium nitrite. Food & Chemical Toxicology, **22**:715–720.

Lijinsky W, Reuber MD (1980). Tumours induced in Fischer 344 rats by the feeding of disulfiram together with sodium nitrite. Food Cosmet Toxicol, **18**:85–87.

Lijinsky W, Taylor HW (1977): Feeding tests in rats on mixtures of nitrite with secondary and tertiary amines of environmental importance. Food Cosmet Toxicol, **15**:269–274.

Lijinsky W, Greenblatt M, Kommineni C (1973a). Brief communication: feeding studies of nitrilotriacetic acid and derivatives in rats. J Natl Cancer Inst, **50**:1061–1063.

Lijinsky W, Taylor HW, Snyder C, Nettesheim P (1973b): Malignant tumours of liver and lung in rats fed aminopyrine or heptamethyleneimine together with nitrite. Nature, **244:**176–178.

Lindinger H, Scheildleder A (2004): Nitrate in groundwater: EROWATERNET European Environment Agency Indictor Fact Sheet 2004.

Lione A (1987): Ionising radiation and human reproduction. Reprod. Toxicol.**1:** 3-16

Lioy P (1990): Assessing total human exposure to contaminants: A multidisciplinary approach. Environ. Sci. Technol. **24 (7)**, pp 938–945.

Little J, Elwood J M (1992): Geographical variation. In: Epidemiology and Control of neural tube defects. Eds: Elwood JM, Little J & Elwood JH. Oxford University press pg96-145.

Logothetopoulus J, Scott R.F (1956): Active iodide transport across the placenta of the guinea-pig, rabbit and rat. J Physiol. 1956; **132**:365–371.

Longnecker MP, Daniels JL (2001): Environmental contaminants as etiologic factors for diabetes. Environ Health Perspective **109**:871–876.

Lord, E.I., Shepherd, M., Silgram, M., Goodlass, G., Gooday, R., Anthony, S.G., Davison, P. & Hodgkinson, R. (2006): Interim report on Defra project NIT18: "Investigating the Effectiveness of NVZ Action Programme Measures: Development of a Strategy for England"

Luca, D., Luca, V., Cotor, F.L. and Raileanu, L. (1987): In vivo and in vitro cytogenic damage induced by sodium nitrite. Mutat. Research, **189,** 333-339.

Luckey, T. D. (1975): Hormology with inorganic compounds. In Heavy Metal toxicity, Safety and Hormology Supplement Vol 1 ed. (T. D. Luckey, B. Venugopal, and D. Hutcheson, Eds.), pp. 81–121. George Thieme Publishers, Stuttgart, Germany.

Luckey, T. D. (1980): Hormesis with Ionizing Radiation. CRC Press, BocaRaton, Florida.

Lundberg JO, Weitzberg E, Lundberg JM et al (1994): Intragastric oxide production in humans: measurements in expelled air. Gut (1994) **35**:1543-6

Lundberg JO and Govoni M (2004): Inorganic nitrate is a possible source for systemic generation of nitric oxide. Free Radic Biol Med (2004) **37**: 395-400

Lundberg JO, Weitzberg E (2005): NO Generation from nitrite and its role in vascular control. Arterioscler Thromb Vasc Biol (2005). **5**: 915-22

Lundberg JO, Weitzberg E (1998): No-enzymatic nitric oxide production in humans. Nitric Oxide (1998) **2**:1-7

Lutynski R, Steczek W, Kroch S (1996): The concentrations of nitrates and nitrites in food products and environment and the occurrence of acute toxic methaemoglobinemia. Przegl Lek **53(4)**351-355.

Macera C, Powell K (2001): Population attributable risk: implications of physical activity dose. J. Medicine in Sports & Exercise S635-639.

Mackerras DE, Eastman CJ (2012): Estimating the iodine supplementation level to recommend for pregnant and breastfeeding women in Australia. Med J Aust **197**:238–242.

Maekawa A, Odashima S (1975): Induction of tumors of the nervous system in the ACI/N rat with 1-butyl-1-nitrosourea administered transplacentally, neonatally, or via maternal milk. Gann, **66:** 175–183.

Maekawa A, Ishiwata H, Odashima S (1977): Transplacental carcinogenesis and chemical determination of 1-butyl-1-nitrosourea in stomach content after simultaneous oral administration of 1-butylurea and sodium nitrite to ACI/N rats. Gann**, 68**:81–87.

Maekawa A, Ogiu T, Onodera H et al. (1982): Carcinogenicity studies of sodium nitrite and sodium nitrate in F-344 rats. Food Chem Toxicol, **20**:25–33.

MAFF (1992): Ministry of Agriculture, Fisheries and Food: Nitrate, nitrite and N-nitroso compounds in food: Second Report. Food Surveillance Paper No.32. Ministry of Agriculture, Fisheries and Food, Great Britain 1992

MAFF (1991): Ministry of Agriculture, Fisheries and Food: Code of good agricultural practice for the protection of water. Environmental Matters.

MAFF (1993): Ministry of Agriculture, Fisheries and Food: Solving the nitrate problem. Progress in research and development. Environmental Matters series pg. 37.

Mannix, M. (2006): Large E.coli 0157 outbreak in Ireland, October-November 2005. Notes from Multi Agency Outbreak Control Team. http://www.eurosurveillance.org/ew/2005/051222.asp#3 (accessed 17.01.11)

Manassaram L, Becker L, Moll D (2006): A review of nitrates in drinking water: Maternal exposure and adverse reproductive and developmental outcomes. Env. Health Persp. Vol. 114, **No.3**, 2006

Manassaram D; Backer L; Messing R; Fleming L, Luke B; Monteilh C (2010): Nitrates in drinking water and methemoglobin levels in pregnancy: a longitudinal study. Environmental Health (2010): **60** pg. 1-12

Markel E, Nyakas C, Ormai S (1989): Nitrate induced changes in sensorimotor development and learning behaviour in rats. Acta Physiol. Hung, **74,** 69-75.

Marletta MA, Yoon PS, Iyengar R, Leaf CD, Wishnok JS (1998): Macrophages oxidation of L-arginine to nitrate and nitrate - nitric oxide is an intermediate. Biochemistry **27**, 8706 - 8711.

Marmot, M (2010). Fair Society, Health Lives: Strategic Review of Health Inequalities in England. London. (www.marmotreview.org). Accessed 1/2/14

Mascher F, Marth E (1993): Metabolism and effect of nitrates. Cent Eur J Public Health, **1**:49–52.

Mason J.B, Lofti M, Dalmiya N, Sethuraman K, Deitchler M (2001): The Micronutrient Report. Current progress and trends in the control of vitamin A; iodine; and iron deficiencies. Ottawa, Canada, Micronutrient Initiative, 2001.

Maticic B (1999): The impact of agriculture on groundwater quality in Slovenia: Standards and Strategy: Agriculture Water Management **40**; 235-247

Maynard R, Cameron K, fielder R, Mcdolnald A, and Wadge A (1995): Setting air quality standards for carcinogens: an alternativeto mathematical quantitative risk assessment- a discussion paper. Human Exposure and Toxicology **14** 175-186

McAuley L, Tugwell P, Moher D (2000): Does the inclusion of gray literature estimates of intervention effectiveness reported in meta- analysis? Lancet **356**:1228-31, (2000).

McCormick, M.C., Gortmaker, S.L., and Sobol, A.M (1990): Very low birthweight children: Behaviour problems and school difficulties in a national sample. Journal of Paediatrics (1990), 117:687-93.

McGuire J, Galloway R (1994): Enriching lives: Overcoming vitamin and mineral malnutrition in developing countries. Washington DC: World Bank; 1994.

McKnight, G., Smith, L.M., Drummond, R.S., Duncan, C.W., Golden, M.N.H. and Benjamin, N (1997): The chemical synthesis of nitric oxide in the stomach from dietary nitrate in man. Gut., **40**, 211-214.

McKnight, G.M., Duncan, C.W., Leifert C. and Golden, M.H. (1999): Dietary nitrate in man: friend or foe? British Journal of Nutrition, **81,** 349-358.

Mcleod L, Ray J.G (2002): Prevention and detection of diabetic embryopathy. Community Genetics, **5:** 33 39.

Meeker JD, Barr DB, Hauser R (2009): Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men. Reprod Toxicol **27**:155–160

Meeker JD, Sathyanarayana S, Swan SH (2009): Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos Trans R Soc Lond B Biol Sci364:2097–2113.

Meeker J.D (2010): Exposure to environmental endocrine disrupting compounds and men's health. Maturitas **66**:236–241.

Meeker JD, Stapleton HM (2010): House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ Health Perspect **118**:318–323.

Melnick R, Lucier G, Wolfe M, Hall R, Stancel G, Prins G, Gallo M, Reuhl K, Shuk-Mei Ho, Brown T, Moore J, Leakey J, Haseman J, Kohn M (2002): Summary of the National Toxicology Program's report of the endocrine disruptors low-dose peer review. Environ Health Perspect **110**:427–431.

Mensinga T; Spijers J; Meulenbelt J (2003): Health Implications of Exposure to Environmental Nitrogenous Compounds; Toxicol Rev: **22 (1):** 41-51.

Meybeck M; Helmer R (1996): Surface exchange of odd nitrogen oxide. In: The terrestrial Nitrogen cycle as influenced by Man. (Ed) Kohler W

Michael D (2008): Doubt is their product: How industry's assault on science threatens your health. Oxford University Press, New York.

Miettinen O (1974): Proportion of disease caused or prevented by a given exposure, trait, or intervention. Am. J. Epidemiol. **99**:325-332.

Mirvish SS, Greenblatt M, Kommineni VR (1972): Nitrosamide formation in vivo: induction of lung adenomas in Swiss mice by concurrent feeding of nitrite and methylurea or ethylurea. Journal of National Cancer Institute, **48**:1311–1315.

Mirvish SS, Pelfrene AF, Garcia H, Shubik P (1976): Effect of sodium ascorbate on tumor induction in rats treated with morpholine and sodium nitrite, and with nitrosomorpholine. Cancer Letters, **2**:101–108.

Mirvish SS, Bulay O, Runge RG, Patil K (1980): Study of the carcinogenicity of large doses of dimethylnitramine, N-nitroso-L-proline, and sodium nitrite administered in drinking water to rats. Journal of National Cancer Institute **64**:1435–1442.

Mirvish SS, Salmasi S, Cohen SM, Patil K, Mahboubi E (1983): Liver and forestomach tumors and other forestomach lesions in rats treated with morpholine and sodium nitrite, with and without sodium ascorbate. Journal of National Cancer Institute **71**:81–85.

Miyashita C, Sasaki S, Saijo Y, Washino N, Okada E, Kobayashi S, Konishi K; Kijiwara J; Todaka T; Kishi R (2011): Effects of prenatal exposure to dioxin-like compounds on allergies and infections during infancy. Environmental Research 111**(4)**:551–558.

MMWR (1996): Morbidity and Mortality Weekly Report: Spontaneous abortions possibly related to ingestion of nitrate – contaminated well water – LaGrange County, Indiana, 1991-1994. MMWR 45 **(26):** 569 –72 (1996)

MMWR (1993): Morbidity and Mortality Weekly Report: Methaemoglobinemia in an infant – Wisconsin 1992. MMWR **42**(12) 217-219(1993)

Moher D, Liberati A, Tetzlaff J, Altman DG (2009): PRISMA Group: Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. Journal of Clinical Epidemiology; 62:1006-12.

Mokhtar NM, el-Aaser AA, el-Bolkainy MN, Ibrahim HA, Badr El-Din NK, Moharram NZ (1988): Effect of soybean feeding on experimental carcinogenesis–III. Carcinogenicity of nitrite and dibutylamine in mice: a histopathological study. Eur J Cancer Clin Oncol, **24:**403–411.

Moller H, Landt J, Pedersen E (1989): Endogenous nitrosation in relation to nitrate exposure from drinking water and diet in a Danish rural population. Cancer Research 1989; **49,** 3117 – 3121X

Moller, H. (1995): Adverse health effects of nitrate and its metabolites: epidemiological studies in humans. In: Health aspects of nitrate and its metabolites (particularly nitrite). Proceedings of an international workshop, Bilthoven (Netherlands), 8- 10 November 1994. Strasbourg, Council of Europe Press, pp. 255-268.

Moller H, Landt J, Jensen P, Pedersen E, Autrup H, Jensen OM (1989): Nitrate exposure from drinking water and diet in a Danish rural population. Int J Epidemiol, **18**:206–212.

Mons M; van der Wielen J; Blokker E; Sinclair M; Hulshof K; F. Dangendorf F; Hunter P; Medema G (2007): Estimation of the consumption of cold tap water for microbiological risk assessment: an overview of studies and statistical analysis of data. Journal of water and health. **Vol. 05, Suppl. 1** pg 151-169.

Montague P (2004): Reducing the harm associated with risk assessment. Environmental Impact Assessment Review **24**:733-748.

Morales-Varela M (1994): Nitrate Contamination of drinking Water Sources. In: Health aspects of nitrate and its metabolites (particularly nitrite). Proceedings of an international workshop, Bilthoven (Netherland), 8-10 November 1994, Strasbourg, Council of Europe Press, pp. 115-123.

Morales -Varela M, Llopis-Gonzalez A, Tejerizo-Perez ML (1995): Impact of nitrates in drinking water on cancer mortality in Valencia, Spain. Eur. J. Epidemiol. **11(1):** 15-21.

Moraleas-Varela M, Llopis-Gonzalez A, Tejerizo-Perez ML, Ferrandiz-Ferragud J (1993): Concentration of nitrates in drinking water and its relationship with bladder cancer. J. Environ. Pathol. Toxicol. Oncol. **12(4):**229-36.

Morgan M, Henrion M (1990): Uncertainty: A guide to dealing with uncertainty in quantitative risk and policy analysis. New York Cambridge Press.

Morton W.E (1971): Hypertension and drinking water constituents in Colorado. Am J Public Health **61**:1371–1378.

Morgenstren H (1982): Uses of ecologic analysis in epidemiological research. Am. J. Public Health **72**:1336-1344

MPPP (1999): Massachusetts Precautionary Principle Project. Risk Assessment & Risk Management, Boston, 1999.

MSS (1980): Ministry of Supply and Services, Quebec: Guidelines for Canadian drinking water quality 1980.

Mueller RL, Hagel HJ, Wild H, Ruppin H, Domschke W (1986): Nitrate and nitrite in normal gastric juice: Precursors of the endogenous N-nitroso compounds. Oncology, **43**, 50-53.

Mueller RL, Hagel HJ, Greim G, Ruppin H, Domschke W (1983): Endogenous synthesis of carcinogenic N-nitroso- compounds, bacterial flora and nitrite formation in the healthy human stomach. Hygene B, **178**:297-315.

Mueller BA, Newton K, Holly EA, Preston-Martin S (2001): Residential water source and the risk of childhood brain tumors. Environ Health Perspective, **109:**551–556.

Mueller BA, Searles Nielsen S, Preston-Martin S, Holly EA, Cordier S, Filippini G, Peris-Bonet R, Choi NW (2004): Household water source and the risk of childhood brain tumours: results of the SEARCH International Brain Tumor Study. Int J.Epidemiol, **33**:1209–1216.

Muhrer M. E, Garner G. B, Pfander W. H (1959): The effect of nitrate on reproduction and lactation. J Animal Science 1959; 15: 1291-2

Mukhapadhyay S, Ghosh D, Chatterjee A, Sinha S, Tripathy S, Chandra A.K (2005): Evaluation of possible goitrogenic and anti-thyroidal effects of nitrates, a potential environmental pollutant. Indian J. physiol Phamacol. **Vol. 49, Issue3**, pp 243-288.

Munro I. C, Krewski D. R (1981): Risk assessment and regulatory decision-making. Food Cosmet. Toxicolo. **19**: pp 549-60.

Murray C, Lopez A. (1996): The Global burden of disease. Geneva, World Health Organisation, Harvard school of Public Health, World Bank.

Nadav D, Dani F (2006): Reconstructing data: evidence-based medicine and evidence-based public health in context. Dynamis **26**:287–306.

Nagel SC, vom Saal FS, Welshons W.V (1999): Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. J Steroid Biochem Mol Biol **69**:343–357

NAS (1977): National Academy of Sciences (NAS), Safe Drinking Water Committee, 1977. Drinking Water and Health. Washington, D.C.

NAS (1978): National Academy of Sciences. Environmental Studies Board, 1978. Nitrates: An Environmental Assessment, Washington, DC.

NAS (1995): National Academy of Sciences, 1995 Board on Environmental Studies and Toxicology. Nitrate and Nitrite in Drinking Water. Washington, DC.

NAS (1981): The Health effect of Nitrates, Nitrite and N-nitroso compounds. National Academy of Science, Washington DC

NAS (1981b): The health effects of nitrate, nitrite, and N-nitroso compounds. Part 1 of a two-part study by the Committee on Nitrite and Alternative Curing Agents in Food. Report by the US National Research Council, Washington DC, National Academy Press.

NAS (1995): Nitrate and Nitrite in drinking water. National Academy of Science, Washington DC

NAS (1981): National Academy of Sciences. The health effects of nitrates, nitrites and n-nitroso compounds. Washington DC 1981.

NERRI (1991): Water Mission Reports on participation of National Environmental Engineering Research Institute, Nigpur, India.

NERC (2002): Natural Environment Research Council. An aquifer guide and a pathogen risk assessment toolbox. Environmental Health Practitioners guide, provided by the British Geological Survey to the Chartered Institute of Environmental Health. http://www.cieh.net/dload/pws/pdf/C10_pathogen_migration.pdf (accessed 1/9/13)

Neutra R, Delpizzo V (2002): Transparent Democratic Foresight Strategies in the California EMF Program. Public Health Report Vol. **117**: 553-563.

New York State Department of Environmental Conservation Air Resources (2000): State-wide Air Quality Trends 1995-1996, Air Quality Report, Air Quality Trends 1-9.

Nixon S (1999): Groundwater quality and quantity in Europe. European Environmental Agency, pg.18

Nolan B.T, Ruddy B.C, Hitt K.J (1997): Risk of nitrate in Groundwater of the United States: a national perspective. Environ. Sci Technol. **31**: 2229-2236.

Northridge ME (1995): Annotation: public health methods-attributable risk as a link between causality and public health action. Am. J. Public Health. **85:** No 9; 1202-1203.

Nosé V. (2010): Familial follicular cell tumors: classification and morphological characteristics. Endocrine Pathology, 21:219–226

NRC (1983): National Research Council (1983): Risk assessment in the Federal Government: Managing the process. National Academy Press.

NRC (1977): National Research Council: Drinking water and health. NRC Washington D.C 1977.

NRC (1978): National Research Council: Panel on Nitrates: An environmental Assessment.

NRC (2008): National Research Council. Science and Decisions: Advancing Risk Assessment. National Academy of Science, 2008.

NRC (2005) National Research Council: Health Implications of Perchlorate Ingestion. Washington, DC: National Academy Press; 2005.

NTP (2001): National Toxicology Program (NTP)'s report of the endocrine disruptors low dose peer review. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

OECD (1986): Water pollution by fertilizers and pesticides. Organization of Economic Corporation and Development 1986

Oenema O, Boers, P.C.M. and Willems, W.J (1998): Leaching of nitrate from agriculture to groundwater: The effect of policies and measures in the Netherlands. Environmental Pollution, **102**, 471-478.

Office of Science and Technology Policy (1985): Chemical carcinogens: a review of the science and its associated principles. Federal Register 50(50): pp 10372-442

Ohshima H, Bartsch H (1981): Quantitative estimation of endogenous nitrosation in humans by monitoring N-nitrosoproline excreted in the urine. Cancer Research, **41**:3658–3662.

Ohshima H, Bartsch H (1994). Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat Res, **305**:253–264.

Ohshima H, Bandaletova TY, Brouet I, Bartsch H, Kirby G, Ogunbiyi F, Vatanasapt V, Pipitgool V (1994): Increased nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase induced in hamsters infected with liver fluke (Opisthorchis viverrini). Carcinogenesis, **15**:271–275.

Olaleye O, Ekrikpo U, Moorthy R, Lyne O, Wiseberg J, Black M, Mitchell D (2011): Increasing incidence of differentiated thyroid cancer in South East England: 1987-2006. European Archives of Otorhinolaryngol 2011; **268(6)**:899-906.

OME (2001): Office of Migrant education: Comprehensive Needs Assessment. Materials adopted from Planning and conducting need assessment: a priactical guide 1995.

ONS (2013): Office of National Statistics. 2011 Census: Population and household estimates for the United Kingdom, March 2011. www.ons.gov.uk. Accessed 2, October 2013.

ONS (2016): Office of National Statistics UK. Cancer Registration Statistics, England (2014).

O'Riordan T, Cameron J (1994): The history and contemporary significance of the precautionary principle. In: Interpreting the precautionary principle: London Earthscan Publications 1994.

Ormandy D, Braubach M (2011): Introduction: In: Environmental burden of disease associated with inadequate housing. Methods for quantifying health impacts of selected housing risks in the WHO European Region. Pg. 1-3. Edited by: Braubach M., Jacobs, D.E., Ormandy D.

Otake M, Shull W.J (1998): Radiation related brain damage and growth retardation among prenatally exposed atomic bomb survivors. International Journal of Radiation Biology**74 (2):** 159-71

Otake M, Shull W.J (1984): In utero exposure of a bomb radiation and mental retardation; a reassessment. British Journal Radiolology: **57**: 409-414.

Ott W, Steinemann A, Wallace L (2007): Exposure analysis. Taylor & Francis Publication, p. 8-13.

Oztasan N (2012): The effects of added drinking water nitrate on plasma leptin, insulin and thyroid hormone concentrations in rats, Journal Animal. Vet. Adv. **11,** 3050–3053.

Pacheco, J. and Cabrera, S. (1997): Groundwater contamination by nitrates in the Yucatan Peninsula, Mexico. Hydrogeology Journal, 5, 47-53.

Palli D, Russo A, Decarli A (2001): Dietary patterns, nutrient intake and gastric cancer in a high risk area of Italy. Cancer Causes Control, **12**:163–172.

Pandav CS (1996): The economic benefits of the elimination of IDD. In: Hetzel BS, Pandav CS,eds. S.O.S. for a billion. The conquest of iodine deficiency disorders. New Delhi, Oxford University Press, 1996: 129–145.

Paneth, N (1986): Recent trends in preterm delivery rates in the United States. INSERM Colloquia: Prevention of Preterm Birth (1986) **138**:15-30.

Parascandola M, Weed DL (2001): Causation in epidemiology. J Epidemiol Community Health 2001; **55**: 905-12.

Parkin D (2011a): The fraction of cancer attributable to lifestyle and environmental factors in the UK in 2010. British Journal of Cancer (2011): 105, S2-S5.

Parkin D (2011b): Tobacco attributable cancer burden in the UK in 2010. British Journal of Cancer (2011): **105**, S6-S13.

Parkin D (2011c): Cancer attributable to consumption of alcohol in the UK in 2010. British Journal of Cancer (2011): **105,** S14-S18.

Paustenbach D, Galbraith D. (2006): Biomonitoring: is body burden relevant to public health? Regul Toxicol Pharmacol **44(3)**:249–261.

Paustenbach D.J (1989): The risk assessment of environmental hazards. New York. John Wiley & Sons Ltd.

Paternain JL, Domingo JL, Llobet, Corbella J (1998): Embryology and teratogenic effects of Aluminum nitrate in rats upon oral administration. Teratology **38 (3**) 253-7

Pearce EN, Lazarus JH, Smyth P.P, He X, Dall'amico D, Parkes AB, Burns R, Smith DF, Maina A, Bestwick J.P, Jooman M, Leung AM, Braverman LE (2010): Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women. J.Clin. Endocrinol. Metab 95:3207–3215.

Pearce EN, Andersson M, ZimmermanM. B (2013): Global Iodine Nutrition: Where Do We Stand in 2013? Thyroid: **Volume 23, Number 5**, 2013.

Pearce EN, Pino S, He X, Bazrafshan H.R, Lee SL, Braverman LE (2004): Sources of dietary iodine: bread, cows' milk, and infant Formula in the Boston area. J. Clin. Endocrinol. Metab **89**:3421–3424.

Pedersen I B, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Rasmussen LB.(2006): Increase in incidence of hyperthyroidism predominantly occurs inyoung people after iodine fortification of salt in Denmark. J.Clinical. Endocrinol. Metab 2006; **92**:3830–34.

Pedersen I.B, Knudsen N, Carle A, Vejbjerg P, Jorgensen T, Perrild H, Ovesen L, Rasmussen L.B, Laurberg P (2011): A cautious iodization program bringing iodine intake to a low recommended level is associated with an increase in the prevalence of thyroid autoantibodies in the population. Clinical. Endocrinol. 2011; **75**:110–16.

Pedersen I.B, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Rasmussen L.B.(2007): An increased incidence of overt hypothyroidism after iodine fortification of salt in Denmark: a prospective population study. J. Clin. Endocrinol. Metab. 2007; **92**:3122–27.

Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R (2013): Worldwide increasing incidence of thyroid cancer: Update on epidemiology and risk factors. Journal of cancer epidemiology **2013**; 2013: 965212.pgs1-10

Petersen J, Alexander G (2002): The context for Needs assessment: past, present and future. IN: Needs assessment in public health: A practical guide for students and professional. Chapter 1, pg.1-10. Kluwer Academic Publishers, New York 2001, New York.

Peterson E, De P, Nuttal R (2012): BMI, diet and female reproductive factors as risks for thyroid cancer: a systematic review. PLoS ONE 2012; **7(1):**e29177

Phillips CV, Goodman KJ (2004): The missed lessons of Sir Austin Bradford Hill. Epidemiol Perspect Innov 2004; **1**: 3.

Phillips D I (1997): Iodine, milk, and the elimination of endemic goitre in Britain: the story of an accidental public health triumph. J. Epidemiology & Community Health 1997; **51**: 391-393.

Pless I (2003): Expanding the precautionary principle. Internationl Journal Prevention. **9**; 1-2

Pliss GB, Frolov AG (1991): Sodium nitrate as a possible promoter of bladder carcinogenesis in rats. Vopr Onkol, **37**:203–206.

Pobel D, Riboli E, Cornée J, Hémon B, Guyader M..(1995): Nitrosamine, nitrate and nitrite in relation to gastric cancer: a case-control study in Marseille, France. Eur J Epidemiol **11**:67–73.

Poland B, Coburn D, Robertson A, Eakin J (1998): Wealth, equity and health care: A critique of a population health perspective on the determiannts of health. Social science & Medicine 46: 785-798.

Ponsioen, J. (1962): Social welfare policy: Contributions to theory. The Hague, the Netherlands: Mouton.

Powlson DS, Addiscott TM, Benjamin N, Cassman KG, de Kok TM, van Grinsven H, L'Hirondel JL, Avery AA, van Kessel C (2008): When does nitrate become a risk for humans? Journal Environ.Quality **37**(2):291-5.

Prassad, J. (1983): Effect of high nitrate diet on thyroid glands in goats. Indian Journal of animal sciences (New Delhi), **53,**791-794.

Preston-Martin S, Correa P (1989): Epidemiological evidence for the role of nitroso compounds in human cancer. Cancer Survey 1989; **8**: 459-473.

Prospero, J .M, Savoie, D.L (1989): Effect of continental sources of nitrate concentrations over the Pacific Ocean. Nature, **339**(6227):687-689.

Prüss A, Kay D, Fewtrell L, Bartram J. (2002): Estimating the burden of disease from water, sanitation and hygiene at a global level. Environmental Health Perspective 110:537–542.

Pruss –Ustun A, Mathers C, Corvalan C, Woodward A. (2003): Introduction and methods: Assessing the environmental burden of disease at national and local levels. World Health Organisation Publications

Pruss-Ustun A, Corvalan C (2006): Preventing diseases through a healthy environment: Towards an estimate of the environmental burden of disease. World Health Organisation Publications, (2006).

Qian M, Wang D, Watkins WE, Gebski V, Yan YQ, Li M, Chen ZP (2005): The effects of iodine on intelligence in children: a meta-analysis of studies conducted in China. Asia Pac J ClinNutr **14**:32–42.

Radikova Z, Tajtakova M, Kocan A, Trnovec T, Sebokova E, Klimes I, Langer P (2008): Possible effects of environmental nitrates and toxic organochlorines on humqan thyroid in highly polluted areas in Slovakia. Thyroid Vol.18, No.3 pg353-362.

Rademacher JJ, Young TB, Kanarek MS (1992): Gastric cancer mortality and nitrate levels in Wisconsin drinking water. Arch Environ Health 1992; **47**: 292-294.

Radomski M.W, Palmer R.M.J Moncada S (1990): An L-arginine/nitric oxide pathway present in human platelets regulates aggregation: Proceedings of the National Academy of Sciences USA, **87**, 5193-5197.

Radomski, M. W., Rees, D.D., Dutra, A. and Moncada, S (1992): S-nitroso-glutathione inhibits platelet activation in vitro and in vivo. British Journal of Pharmacology,**107**,745-749.

Radomski M, Palmer R, Moncada S (1987): Comparative pharmacology of endothelium – derived factor, nitric oxide and prostacylin in platelets. Br. J. Pharmcol. **92,** 181-187.

Rahman GA, Abdulkadir AY, Braimoh KT, Inikori AR (2010): Thyroid cancers amongst goiter population in a Nigerian tertiary hospital: surgical and radiographic perspective. Nigeria J. Med. **19(4)** 432-435).

Rappaport SM, Smith MT (2010): Environment and Disease Risks. Science, **Vol: 330, No.6003**: pp 460–461.

Rasmussen, L.B.; Carle, A.; Jorgensen, T.; Knudsen, N.; Laurberg, P.; Pedersen, I.B.; Perrild, H.; Vejbjerg, P.; Ovesen, L (2008): Iodine intake before and after mandatory iodization in Denmark: Results from the Danish Investigation of Iodine Intake and Thyroid Diseases (DanThyr) study. Br. J. Nutr. 2008, **100**, 166–173.

Rayman M.P, Sleeth M, Walter A, Taylor A (2008): Iodine deficiency in UK women of child-bearing age. Proc.Nutr.Soc 2008; **67:** E399.

Reacher, M., Ludlam, H., Irish, N., Buttery, R., and Murray, V. (1999): Outbreak of gastroenteritis associated with contamination of a private borehole water supply. Communicable Disease and Public Health 1999; **2**: 27-31.

Reacher, M., Ludlam, H., Irish, N., Buttery, R., Murray, V. (1999): Outbreak of gastroenteritis associated with contamination of a private borehole water supply.Communicable Disease and Public Health 1999; 2: 27-31.

Ritter L, Keith S, Sibley, Hall K, Keen P, Mattu G, Linton B (2002): Sources, pathways and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. Journal of toxicology and environmental health, Part A, **65**:1-142.

Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, vom Saal FS (2007): Invivo effects of bisphenol A in laboratory rodent studies. Reprod Toxicol **24:**199–224.

Rivers C.N., Hiscock, K.M., Feast, N.A., Barrett, M.H. and Dennis, P.F. (1996): Use of nitrogen isotopes to identify nitrogen contamination of the Sherwood sandstone aquifer beneath the City of Nottingham, UK. Hydrogeology Journal, **4,** 90-102.

RIVM (1993): Maintenance of the environmental law 1995/1997. The quality of the drinking water in the Netherlands in 1993. National Institute of Public Health and Environmental Protection (RIVM Report No. 731011007).

Roberts W, Abernathy C (1996): Risk assessment: principles and methodologies. In: Toxicology and risk assessment: Principles, methods and applications. Pg 245-270. Eds: Fann A, Chang L.

Robertson, B., Forbes, A., Sinclair, M. & Black, J. (2000a): How well does a telephone questionnaire measure drinking water intake? Aust. N.Z.J. Public Health **24(6)**, 619–622.

Robertson, B., Sinclair, M. & Forbes, A (2000b): The effect of an introductory letter on participation rates using telephone recruitment. Aust. N.Z. J.Public Health **24(5**), 552.

Robertson, B., Sinclair, M. I., Forbes, A. B., Veitch, M., Kirk, M., Cunliffe, D., Willis, J. & airley, C. K. (2002): Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide. Australia. Epidemiol. Infect. **128**, 419–431.

Rockhill B, Newman B, Weinberg C (1998): Use and Misuse of Population Attributable Fractions. Commentary: Am J Public Health. **Vol.88, No1** 15-19.

Roediger W, Deakin, Radcliffe B (1986): Anion control of sodium absorption in the colon. Quaterly J Exp. Physio., **71**, 195-204.

Rogan W (2014): Iodine Deficiency, Pollutant Chemicals, and the Thyroid: New Information on an Old Problem. Paediatrics (2014); **133**; 1163-1166.

Rogan W & Brady M (2009): Drinking water from private wells and risks to children. Committee on Environmental Health and Committee on Infectious Diseases. Paediatrics (2009); **123(6)**; 1599-1605.

Rogers MA, Vaughan TL, Davis S, Thomas DB (1995): Consumption of nitrate, nitrite, and nitrosodimethylamine and the risk of upper aerodigestive tract cancer. Cancer Epidemiol Biomarkers Prev, **4**:29–36.

Romieu I, Weitzenfeld H, Finkelman J (1990): Urban Air Pollution in Latin American and the Caribbean: Health Perspectives. World Health Statistical Quarterly **43(3)**: 153–67.

Ron E, Schneider A. (2006): Thyroid cancer. In: Schottenfeld D, Fraumeni J Jr, editors. Cancer epidemiology and prevention. 3rd ed Oxford, UK: Oxford University Press; 975–994

Rosen L (2013): An intuitive approach to understanding the attributable fraction of disease due to a risk factor. The case of smoking. Int. J. Res. Public Health; 2013, **Vol. 10,** 2932-2943.

Roth A.C, Herkert G.E, Bercz J.P, Smith M.K (1987): Evaluation of the developmental toxicity of sodium nitrate in Long Evans rat. Fundam. Appl. Toxicol. **96,** 668-677.

Roth A.C, Smith M.K (1988): Nitrite - induced iron deficiency in the neonate rat. Toxicol. Appl. Pharmacol**. 96**, 43-55

Rothman K.J, Moore L.L, Singer M.R et al (1995): Teratogenicity of high vitamin A intake. New Eng. J.Med. **333**:1369-73.

Rothman KJ, Greenland S (2005): Causation and causal inference in epidemiology. Am. J. Public Health 2005; **95(Suppl. 1):**S144-50.

Rudell W.S, Bone E.S, Hill, M.J. Walters C.L (1978): Pathogenesis of gastric cancer in pernicious anaemia. Lancet, 1,521-523.

Rutter, M., Nichols, G.L., Swan, A., and De Louvois, J. (2000): A survey of the microbiological quality of private water supplies in England. Epidemiology and Infection (2000), **124,** 417-425.

Ryan, B. C., Hotchkiss, A. K., Crofton, K. M. & Gray, L. E (2010): In Utero and lactational Exposure to Bisphenol A, In Contrast to Ethinyl Estradiol, Does Not Alter Sexually Dimorphic Behavior,Puberty, Fertility, and Anatomy of Female LE Rats. Jr Toxicol. Sci. **114**, 133–148

Sagai M, Ichinose T (1991): Experimental study on lipid peroxidase formation and tumor promoting effects of nitrogen oxide and ozone. In Oxidative damage and repair. Edited by Davies KTA. Pergammon: Oxford; 1991:511-516.

Said, B., Wright, F., Nichols, G.L., Reacher, M., and Rutter, M. (2003). Outbreaks of infectious disease associated with private drinking water supplies in England and Wales 1970-2000. Epidemiology and Infection (2003), **130 (3),** 469-479.

Saleh, Z.A., Brunn, H., Paetzold, R. and Hussein, L (1998): Nutrients and Chemical residues in an Egyptian total mixed diet. Food Chemistry, **63**, 535-541.

Salvemini D, de Nucci G, gryglewski R, Vane J (1989): Human neutrophils and mononuclear cells inhibit platelet aggregation by releasing a nitric oxide-like factor. Proc. Natl. Acad Sci; **86;** 6328 -6332.

Samet JM, Schnatter R, Gibb H (1998): Epidemiology and Risk assessment. Am. J Epidemiology **Vol. 148: No. 10** 929-938

Sand S (2005): Dose-Response Modelling: Evaluation, Application and Development of Procedures for Benchmark Dose Analysis in Health Risk Assessment of Chemical Substances. Karolinska University Press, Stockholm, Sweden 2005.

Sandor J, Kiss I, Farkas O, Ember I (2001): Association between gastric cancer mortality and nitrate content of drinking water: ecological study on small area inequalities. Eur J Epidemiol, **17**:443–447.

Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, the PRISMA-P Group (2015): Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. **British Medical Journal 349**:g7647 doi: 10.1136/bmj.g7647

Scott J (2010): Nitrate Contamination Spreading in California Communities. California Watch, May 15, 2010

Scotish Executive (2006): Private water supplies. Technical Manual, Pg7-13. June 2006.

Schubert C, Knobeloch L, Kanarek MS, Anderson HA. (1999): Public response to elevated nitrate in drinking water wells in Wisconsin. Arch Environ Health **54(4)**: 242–247.

Scragg R K, Dorsch MM, McMichael J, Baghurt PA (1982): Birth defects and household water water supply. Epidemiology studies in the mouth Gambier of South Australia. Med J Australia 1982; **2:** 577 – 9

SEER (2012): Surveillance, Epidemiology & End Results Program. http://seer.cancer/statfacts/html/thyro.html (Accessed 20/1/15).

Seller M (2004): Genetic causes of congenital anomalies and their interaction with environmental factors: In: Review of Environmental risk factors for congenital anomalies. EUROCAT Working Group 2004.

Sen, N.P, Baddoo P.A (1997): Trends in the levels of residual nitrite in Canadian cured meat products over the last 25 years. J.Agric.Food Chem., **45**, 4714-4718.

Shapiro K.B; Hotchkiss J.H; Roe D.A. (1991): Quantitative relationship between oral nitrate reducing activity and the endogenous formation of N- nitroso amino acids in humans. Food and Chemial Toxicology, **29:** 751 –755.

Shaw GM, Malcoe LH (1992): Residential mobility during pregnancy for mothers of infants with or without congenital cardiac anomalies: a reprint. Archives of Environmental Health **47**:236–238. 1992.

Shearer LA, Goldsmith JR, Young C, Kearns OA, Tamplin B.R (1972): Methemoglobin levels in infants in an area with high nitrate water supply. American Journal of Public Health **62(9)** 1174–1180.

Sheehan DM, Willingham E, Gaylor D, Bergeron JM, Crews D (1999): No threshold dose for estradiol-induced sex reversal of turtle embryos: how little is too much? Environmental. Health Perspectives **107**:155–159.

Sheehan DM, vom Saal FS (1997): Low dose effects of hormones: a challenge for risk assessment. Risk Policy Rep **4**:31–39.

Shephard, S.E., Schlatter, C. and Lutz, W.K. (1987): Assessment of the risk of formation of carcinogenic *N*-nitroso compounds from dietary precursors in the stomach. Food and Chemical. Toxicology, **25(1**): 91–108.

Shimokura G, Savitz D, Symanski E (1998): Assessment of water use for estimating exposure to tap-water contaminants. Environmental Health Perspectives: **Vol.106, No.2,** pg 55-59.

Setzer RW, Kimmel CA (2003): The use of NOAEL, benchmark dose and other models for human risk assessment of hormonally active substances. Pure Appl. Chem Vol. 75 Nos 11-12, pp. 2151-2158.

Shore R.E (1995): Epidemiological Data in Risk Assessment – Imperfect but Valuable. American Journal of Public Health. **Vol. 85, No 4:** 474 -6.

Shuval H I, Gruener N (1972): Epidemiological and toxicological aspects of nitrate and nitrite in the environment. Am J Epidemiol.1972; **(62) 8** 1045 –52.

Shuval, H.I. and Gruener, N (1997): Infant methaemoglobinaemia and other health effects of nitrates in drinking water. Prog. Water Tech, **8**, 183-193.

Shephard, S.E (1995): Endogenous formation of N-nitroso compounds in relation to the intake of nitrate or nitrite. In: Health aspects of nitrate and its metabolites (particularly nitrite). Proceedings of an international workshop. Bilthoven (Netherlands), 8-10 November 1994, Strasbourg, Council of Europe Press, pg.137-150.

Shepherd, K.K., and Wyn-Jones, A.P. (1997): Private water supplies and the local authority role: results of the UK national survey. Water Science and Technology, **Volume 35, Issue 11-12,** 1997:pg. 41-45.

Silbergeld E; (1993): Risk Assessment: The perspective and experience of US environmentalists. Environmental Health Perspective **101(2)** 100-104.

Simescu M, Varciu M, Nicolaescu E, Gnat D, Podoba J, Mihaescu M, Delange F(2002): Iodized oil as a complement to iodized salt in schoolchildren in endemic goiter in Romania. Hormone Research, 2002, **58:**78–82.

Simon C, Manzke H, Kay H, Mrowetz G (1964): Occurrence, pathogenesis, and possible prophylaxis of nitrite induced methemoglobinemia. Zeitschr. Kinderheilk **91**: 124-38.

Skeaff, S.A (2011): Iodine deficiency in pregnancy: The effect on neurodevelopment in the child. Nutrients 2011**, 3**, 265–273.

Sleight SD, Sinha DP, Uzoukwu M (1972): Effect of sodium nitrite on reproductive performance of pregnant cows. Journal of American Veterinary Medical Association. **161 (7):** 819-823

Smith A, Datta S, smith G, eds (1997): Nitric oxide: In: Oxford Dictionary of Biochemistry and Molecular Biology, pg 451.

Smith MA, Shah NR, Lobel JS, Hamilton W (1988): Methemoglobinemia and hemolytic anemia associated with Campylobacterjejuni enteritis. Am J Pediatr Hematol Oncol **10:**35–38.

Smyth P, Burns R, Casey M, Ru Jin Huang, Hoffman T, O'Dowd C, Higashino, H., Tabuchi, M., Yamagata, S., Kurita, T.,Miya, H., Mukai, H. and Miya, Y. (2010): Serum nitric oxide metabolite levels in groups of patients with various diseases in comparison of healthy control subjects. J. Med. Sci. **10**: 1-11.

Soto AM, Sonnenschein C (2010): Environmental causes of cancer: endocrine disruptors as carcinogens. Nat Rev Endocrinol.**6**:363–370.

Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO (1995): The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect **103**(Suppl 7):113– 122.

Soto AM, Chung KL, Sonnenschein C (1994): The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human oestrogen-sensitive cells. Environ Health Perspective **102**:380–383.

Spalding RF, Exner M (1993): Occurrence of nitrates in groundwater – a review. J.Environmental Quality **22**:392-402

Spickett J, Brown H, Martison M, Katsherian D (2006): Health risk assessment in Western Australia. Dept of health (2006).

Spiejers DG and Brandt PA (2003): Nitrate (and potential endogenous formation of N-nitroso compounds) in WHO Food Additives series: 50: 2003.

Speijers, G.J.A. et al. (1989): Integrated criteria document nitrate; effects. Appendix to RIVM Report No. 758473012. National Institute of Public Health and Environmental Protection (RIVM Report No. A758473012).

Speijers G. J. A (1994): Different Approaches of Establishing Safe levels for nitrate and nitrite. In: Health aspects of nitrates and its metabolites. Proceedings of international workshops, Bilthoven, Netherlands 1994. Council of Europe 1995.

Spiegelhalder B, Eisenbrand G, Preussmann R (1976): Influence of dietary nitrate on nitrite content of human saliva: possible relevance to in vivo formation of N-nitroso compounds: Food and cosmetics toxicology, **14**, 545- 548.

Stanbury JB, Ermans AE, Bourdoux P, Todd C, Oken E, Tonglet R, Vidor G, Braverman LE, Medeiros-Neto G (1998): Iodine-induced hyperthyroidism: occurrence and epidemiology. Thyroid, 1998, 8:83–100.

Stangl DK, Berry DA. Meta-analysis in medicine and health policy. Marcel Dekker, New York, NY. 2000.

Stebbing, A. R. D. (1982): Hormesis: The stimulation of growth by low levels of inhibitors. Sci. Total Environ. **22**, 213–234.

Stebbing, A. R. D. (1987): Growth hormesis: A by-product of control. Health Physics 52, 543–547.

Steenland, K; Armstrong, B (2006): An overview of methods for calculating the burden of disease due to specific risk factors. Epidemiology 2006, **17**, 512–519.

Steindorf K, Schlehofer B, Becher H, Hornig G, Wahrendorf J (1994): Nitrate in drinking water. A case-control study on primary brain tumours with an embedded drinking water survey in Germany. Int. J. Epidemiol. **23(3):** 451-7.

Stillerman KP, Mattison DR, Giudice LC, Woodruff TJ (2008): Environmental exposures and adverse pregnancy outcomes: a review of the science. Reprod Sci **15**:631–650

Stormshak F, Leake R, Wertz N, Gorski J (1976): Stimulatory and inhibitory effects of estrogen on uterine DNA synthesis. Endocrinology **99**:1501–1511

Strebel, O, Duynisveld, W.H.M., Bottcher, J. (1989): Nitrate pollution of groundwater in Western Europe: Agriculture, Ecosystem and Environment **26**, 189-214.

Stuehr D, Gross S, Sakuma I (1989): Activated murine macrophages secrete a metabolite of arginnine with the bioactivity of endothelium – derived relaxing factor and the chemical reactivity of nitric oxide. J Exp Med. **169**, 1011-1020

Sugiyama K, Tanaka T, Mori H (1979): Carcinogenicity examination of sodium nitrate in mice. Gifu Daigaku Igakubu Kiyo, **27**:1–6.

Sullivan L.A (1989): North Carolina Surveillance of Birth Defects. Raleigh, NC: North Carolina DEHHR, 1989.

Super M, Heese H, MacKenzie D, Dempster WS, duPless J, Ferreira J.J (1981): An epidemiologic study of well water nitrates in a group of south West African Namibian infants. Water Research **15**:1265–1270.

Sund J, Wright M. J, Simon, J (1957): Weeds containing nitrate cause abortion in Cattle. Agronomy Journal 1957; **49**: 278-9.

Sutton H, Roberts P, Winterbourn C, (1976): The rate of reaction of superoxide radical ion with oxyhaemoglobin and methmoglobin. Biochem., J (1976) **155,** 503 - 510.

Swaen G, van Amelsvoort L (2009): A weight of evidence approach to causal inference. Journal of Clinical Epidemiology **62** (2009) 270-277.

Swan SH (2008): Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environmental Research **108**:177–184.

Swan SH, Liu F, Hines M, Kruse RL, Wang C, Redmon JB, Sparks A; Weiss B (2010): Prenatal phthalate exposure and reduced masculine play in boys. Int J Androl **33(2)**:259–269.

Suzuki J, Yagi N, Suzuki S (1984): Photochemical nitrosation of phenol in aqueous nitrate solution. Chem. Parm Bull (Tokyo) 1984; **32**: 2803-2808.

Tahira T, Ohgaki H, Wakabayashi K et al. (1988): The inhibitory effect of thioproline on carcinogenesis induced by N-benzylmethylamine and nitrite. Food Chem Toxicol, **26**:511–516.

Tajtakova M, Semanova Z, Tomkova Z, Szokeova E, Majoros J, Radikova Z, Sebokova E, Klimes I, Langer P. (2006): Increased thyroid volume and frequency of thyroid disorders signs in schoolchildren from nitrate polluted area. Chemosphere, **62**:559–564.

Tannenbaum, S.R., Fett, D., Young, V.R., Land, P.D and Bruce, W.R (1978): Nitrite and nitrate are formed by endogenous synthesis in the human intestine. Science **200,** 1487-1489.

Tannenbaum SR, Sinskey AJ, Weisman M, Bishop W (1974): Nitrite in Human Saliva. It's possible relationship to nitrosamine formation. Journal of National Cancer Institute **53**:79, (1974).

Taylor HW, Lijinsky W (1975a): Tumor induction in rats by feeding heptamethyleneimine and nitrite in water. Cancer Research, **35**:812–815.

Taylor HW, Lijinsky W (1975b): Tumor induction in rats by feeding aminopyrine or oxytetracycline with nitrite. Int J Cancer, **16**:211–215.

Taylor P, Okosieme O, Dayan C and Lazarus J (2014): Impact of iodine supplementation in mild-to-moderate iodine deficiency: systematic review and meta-analysis. European Journal of Endocrinology, (2014) **170**, R1-R15.

Tebbutt, T.H.Y (1998): Principles of Water Quality Control (5th Edition). Butterworth-Heinemann, Oxford.

Teramoto S, Saito R, Shiaru Y (1980): Teratogenic effcts of combined administration of ethylenethiourea and nitrite in mice. Teratology **21(1)** 71-78.

Terblanche, A.P.S. (1991): Health hazards of nitrate in drinking water and possible means of denitrification. Water S.A **17**.77-83.

The Private Water Supply Regulations (1991): Statutory Instrument 1991: No. 2790.

The private Water Supply Regulation (2009): Statutory Instrument 2009: No 3101.

Thomas JO, Ogunbiyi JO (1995): Thyroid cancers in Ibadan, Nigeria (1995): East African Medical Journal; **72(4)**:231-3.

Thouez J-P, Beauchamp Y, Simard A (1981): Cancer and the physicochemical quality of drinking water in Quebec. Soc Sci Med 1981; **15D**: 213-223.

Til, H.P. et al. (1988): Evaluation of the oral toxicity of potassium nitrite in a 13-week drinking-water study in rats. Food chemistry and toxicology, **26(10):** 851-859.

Til, H.P., Kuper, C.F. and Falke, H.E. (1997): Nitrite-induced adrenal effects in rats and the consequences for the no-observed-effect level. Food and Chemical Toxicology, **35**, 349-355:

Tiwari B; Godbole M; Chattopadhyay N; Mandal A; Mithal A (1996): Leaning disabilities and poor motivation to achieve due to prolonged iodine deficiency. American Journal of clinical Nutrition. **63(5)**: 782-786.

Tjeert T, Spiejers J, Meulenbelt J (2003): Health implications of exposure to environmental nitrogenous compounds. Toxicology Review 2003 **22 (1):** 41-51.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K, Gibbs J (2004): Relative potencies and additivity of perchlorate, thiocynate, nitrate and iodide on the

inhibition of radioactive iodide uptake by the human sodium iodide symporter. Thyroid; Vol. 14, **No. 12;** pg 1012-1019.

Toussaint W, Selenka F (1970): Methemoglobinemia formation in infants: A contribution to drinking water hygiene in Rhine. June: 282-4.

Tricker AR (1997): N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. Eur J Cancer Prev, **6**:226–268

Trush M (2008): The Toxicological process. Fundamental principles of toxicology. John Hopkins School of Public Health. Lecture series section A (2008).

Tukker A (2002): The precautionary principle and epidemiology: a contradicto in terminis? J. Epidemiol. Community Health 2002; **56**; 883-4.

Twort, A.C., Ratnayaka, D.D., and Brandt, M.J. (2000): Water Supply (5th Edition): Arnold Publishers, London.

Tyl, R.W; Myers C; Marr M; Sloan C; Castillo N; Veselica M; Seely J; Dimond S, Van Miller J; Shiotsuka R; Beyer D; Hentges S; Waechter J (2008): Two-Generation Reproductive Toxicity Study of Dietary Bisphenol A in CD-1 (Swiss) Mice. Toxicol. Sci. **104**, 362–384.

US Department of Energy (1996): Characterization of Uncertainties in Risk Assessment with Special Reference to Probabilistic Uncertainty Analysis. EH - 413-068/0496.
USDHHS (2004): USA Department of Health & Human Services: The Health Consequences of Smoking: A Report of the Surgeon General; Washington, DC, USA, 2004.

USDHEW (1964): US Department of Health, Education and Welfare: Smoking and Health: Report of the Advisory committee to the Surgeon General of the Public Health Service Washington DC. Public Health Service Publication No.1103

UNICEF (2012): The State of the World's Children 2012: Children in an Urban World. United Nations Children's Fund, New York, NY.

USEPA (1986): U.S Environmental Protection Agency. Guidelines for the health risk assessment of chemical mixtures. Fed Reg. 51: 34014–34025.

USEPA (1987): Estimated national occurrence and exposure to nitrate and nitrite in public drinking water supplies: Washington, DC, US Environmental Protection Agency, Office of Drinking Water.

USEPA (1989): United States Environmental Protection Agency. Toxicity Assessment: In: Risk Assessment Guidance for Superfund Vol.1, Chapter 7. Office of emergency and remedial response. US Environmental Protection Agency. EPA/504/1-89/002 (1989).

USEPA (1990a): United States Environmental Protection Agency. Seminar Publication: Risk assessment, management and communication of drinking water contamination. EPA /560/7-9-008.

USEPA (1990b): Methodology for assessing health risks associated with indirect exposure to combustor emissions, Washington DC EPA/600/6-90/003

USEPA (1990c): Risk Assessment Methodologies: Comparing EPA and state approaches. Washington, DC, EPA570/9-90-012

USEPA (1990d): United States Environmental Protection Agency. Science Advisory Board. Nitrate/Nitrite SAB Briefing Document. February 1, 1990. Washington, DC.

USEPA (1991a): General quantitative risk assessment guidelines for noncancer health effects.

USEPA (1991b): United States Environmental Protection Agency. Guidelines for developmental toxicity risk assessment. EPA/600/FR – 9/001. 1991.

USEPA (1992): United States Environmental Protection Agency. Guidelines for Exposure Assessment. EPA 600Z-92/001

USEPA (1995a): United States Environmental Protection Agency. Integrated Risk Information Services (IRIS), 1995 database.

USEPA (1995b): United States Environmental Protection Agency. Guidance for Risk Characterization. EPA 2/95

USEPA (1996): United States Environmental Protection Agency Guidelines for reproductive toxicity risk assessment. EPA/630/R/009 1996.

USEPA (2000a): United States Environmental Protection Agency. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. Washington, DC: U.S. Environmental Protection Agency.

USEPA (2000b): United States Environmental Protection Agency. Exposure and Human Health Reassessment of 2,3,7,8- Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds: II. Health Assessment for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds, Chapter 8. Dose-Response Modelling for 2,3,7,8-TCDD. SAB, NCEA-I-0835.

USEPA (2000c): United States Environmental Protection Agency Estimated Per Capita Water Ingestion in the United States. EPA-822-R-00-008. USEPA, Office of Water, Washington, DC. http://www.epa.gov/waterscience/drinking/percapita/html. Accessed 25/10/12

USEPA (2004): United States Environmental Protection Agency. An Examination of EPA Risk Assessment Principles and Practice. March 2004.

USEPA (2005): United States Environmental Protection Agency. Guidelines for Carcinogenic Risk Assessment: Risk Assessment Forum. EPA/630/P-03/001F (March 2005).

USEPA (2007): United States Environmental Protection Agency. Chloramines in drinking water. www.epa.gov/lawregs/scwa/mdpb/chloramines-index.cfm. Accessed 2/3/2014.

USCDC (1998): A survey of the quality of water drawn from domestic wells in Nine midwest states. NCEH 97-0265.Atlanta Georgia 1998

NRC (1995): USA National Research Council (1995): Nitrate and nitrite in drinking water subcommittee on Nitrate and Nitrite in Drinking Water, Committee on toxicology. Board on Environmental Studies and Toxicology, Commission on life science. Washington D.C, National Academy Press.

USA Geological Society (1995): Nutrients in groundwater and surface water of the United States: an analysis of data through 1992. Reston, VA: US Geological Survey.

USA Geological Society (1996a): Nitrates in groundwater- assessing the risk. FS-092-96, Reston, Virginia.

USGS (1996b): Nutrients in the Nation's Waters. Identifying problems and progress.

USGS (1996c): Nutrients in the Nation's Waters. Too much of a good thing? USGS circular 1136.

USGS (1999): USA Geological Society.The Quality of nation's waters: Nutrients and pesticides. Circular 1225. US Geological Survey 1999

USOMB (2006): US Office of Management and Budget: Risk Assessment Bulletin. June 2006.

Utiger RD (2006): Iodine nutrition – More is better. New England J Med.2006; **354**:2819–21.

Van Asselt M, Van der Giessen A, Janssen P, Heuberger P, Geuskens (2001): Uncertainty and RIVM's environmental outlooks: Documenting a learning process. National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Lee D-H, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. (2012): Hormones and endocrine disrupting chemicals: low dose effects and non-monotonic dose responses. Endocr Rev; **33(3):**378-455.

Vandenberg LN, Maffini MV, Sonnenschein C, Rubin BS, Soto AM (2009): Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrine Reviews **30**:75–95

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007): Human exposure to bisphenol A (BPA). Reprod Toxicol **24**:139–177.

Vanderpump M.P, Lazarus J.H, Smyth P.P, Laurberg P, Holder RL, Boelaert K, Franklyn JA (2011): Iodine status of UK school girls: a cross-sectional survey. Lancet **377:** pgs. 2007 - 2012.

Vanderpump (2012): Commentary: Iodine deficiency as a new challenge for industrialised countries: A UK perspective. Int. J.Epidemiol. 2012 pg. 1-4

Vandevijvere S, Mourri AB, Amsalkhir S, Avni F, Van Oyen H, Moreno-Reyes R (2012): Fortification of bread with iodizedsalt corrected iodine deficiency in school-aged children, but not in their mothers: a national cross-sectional survey in Belgium. Thyroid **22**:1046–1053.

Van Duijvenboden, Mathijsen A (1989): Intergrated criteria document Nitrate. Bilthoven. RIVM Report No. 758473012

Van Grinsven , H. , Ward , M. H ., Benjamin , N. and de Kok , T. M. C. M.(2006): Does the evidence about health risks associated with nitrate ingestion warrant an increase of the nitrate standard for drinking water? Commentary: Environmental Health: a Global Access Science Source. **5**:26. Pg 1-6. Biomedical Central Open Access.

Van Grinsven H, Rabl A, de Kok, T. M. (2010): Estimation of incidence and social cost of colon cancer due to nitrate in drinking water in the EU: Quantitative cost–benefit assessment. Environmental Health, **9(58).**

Van der Sluijs J; Risbey J; Kloprogge P; Ravetz J; Funtowicz S; Quintana S; Pereira A; De Marchi B; Petersen A; Janssen P; Hoppe R; Huijs S (2003): RIVM/MNP Guidance for uncertainty assessment and communication. Detailed Guidance, University of Utrecht 2003.

Van Leeuwen JA, Waltner-Toews D, Abernathy T, Smit B, Shoukri M (1999): Associations between stomach cancer incidence and drinking water contamination with atrazine and nitrate in Ontario (Canada) agroecosystems, 1987–1991. Int J Epidemiol, **28**:836–840.

Van Maanen JMS, van Dijk A, Mulder K, de Baets MH, Menheere PCA, van der Heide D, Mertens PLJM, Kleinjans JCS (1994): Consumption of drinking water with high nitrate levels causes hypertrophy of the thyroid. Toxicology Letters **72**:365–374.

Van Mil NH, Tiemeier H, Bongers-Schokking JJ, Ghassabian A, Hofman A, Hooijkaas H, Jaddoe VW, de Muinck Keizer-Schrama SM,Steegers EA, Visser TJ et al (2012): Low urinary iodine excretion during early pregnancy is associated with alterations in executive functioning in children. Journal of Nutrition, 2012, **142**: 2167–2174.

Vermeer, I.T.M. and Van Maanen, J.M.S. (2001): Nitrate exposure and the endogenous formation of carcinogenic nitrosamines in humans. Rev. Environ. Health, 16(2): 105–116

Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Carle A, Pedersen IB, Rasmussen LB, Ovesen L, Jorgensen T.(2009): Lower prevalenceof mild hyperthyroidism related to a higher iodineintake in the population: a prospective study of a mandatory iodization programme. Clinical Endocrinology 2009; **71:**440–45.

Vejbjerg P, Knudsen N, Perrild H, Carle A, Laurberg P, Pedersen IB, Rasmussen LB, Ovesen L, Jorgensen T (2007): Effect of a mandatoryiodization program on thyroid gland volume basedon individuals' age, gender, and preceding severity ofdietary iodine deficiency: a prospective, population-based study. J Clinical Endocrinology Metabolism 2007; **92**:1397–401.

Vermeer IT, Pachen DM, Dallinga JW et al. (1998): Volatile N-nitrosamine formation after intake of nitrate at the ADI level in combination with an amine-rich diet. Environ Health Perspect, **106**:459–463.

Vermeer IT, Moonen EJ, Dallinga JW, Kleinjans JC, van Maanen JM (1999): Effect of ascorbic acid and green tea on endogenous formation of N-nitrosodimethylamine and N-nitrosopiperidine in humans. Mutat Res, **428:**353–361.

Vermeer IT, Engels LG, Pachen DM, Dallinga JW, Kleinjans JC, van Maanen JM (2001): Intragastric volatile N-nitrosamines, nitrite, pH, and Helicobacter pylori during long-term treatment with omeprazole. Gastroenterology, **121**:517–525.

Vladeva S, Gatseva P, Gopina G (2000): Comparative Analysis of results from studies of goitre in children from Bulgarian villages with nitrate pollution of drinking water in 1995 and 1998. Central European Journal of Public Health, No**. 3** vol. 8, p179-181

Volkmer BG, Ernst B, Simon J, Kuefer R, Bartsch G Jr, Bach D, Gschwend JE (2005): Influence of nitrate levels in drinking water on urological malignancies: a community-based cohort study. Br J Urol Int, **95**:972–976.

Vom Saal FS, Bronson FH (1978): In utero proximity of female mouse fetuses to males: effect on reproductive performance during later life. Biol Reprod. 1978 Nov; **19(4):**842-53.

Vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV (1997): Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci U S A. 1997**, 94(5):**2056-61.

Vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, Farabollini F, Guillette Jr LJ, Hauser R, Heindel JJ, Ho SM, Hunt PA, Iguchi T, Jobling S, Kanno J, Keri RA, Knudsen KE, Laufer H, LeBlanc GA, Marcus M, McLachlan JA, Myers JP, Nadal A, Newbold RR, Olea N, Prins GS, Richter CA, Rubin BS, Sonnenschein C, Soto AM, Talsness CE, Vandenbergh JG, Vandenberg LN, Walser-Kuntz DR, Watson CS, Welshons WV, Wetherill Y, Zoeller RT (2007): Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol **24**:131–138.

Vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Parmigiani S, Welshons WV (1997): Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. Proc Natl Acad Sci USA **94:**2056–2061.

Von Krauss M, Martuzzi M (2006): Integrating Uncertainty to Integrated Assessment: INTARESE Work Package 1.5 (unpublished).

Von Krauss M (2005): Uncertainty in policy relevant science. PhD Thesis, Institute of Environment and Resources, Technical University of Denmark. www.er.dfu.dk/publications/fulltext/2005/mr2005-202.pdf Assessed 27/6/07.

Vulsma, T., Gons, M.H. and de Vijlder, J.J. (1989): Maternal–fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. New England Journal of Medice, **321(1)**: 13–16.

Wacholder S, Benichou J, Heineman E.F, Hartge P, Hoover RN (1994): Attributable risk: advantages of a broad definition of exposure. American Journal of Epidemiology. 140:303-309.

Wagner, D.A., Schultz, D.S., Deen, W.M., Young, V.R. and Tannenbaum, S.R. (1983): Metabolic fate of an oral dose of 15N-labeled nitrate in humans: effect of diet supplementation with ascorbic acid. Cancer Res., 43: 1921–1925.

Wakeford R, Mcelvenny D (2007): From Epidemiological association to causation. Occupational Medicine **57**:464-465 (Editorial).

Walker R (1990). Nitrates, nitrites and N-nitroso compounds: a review of the occurrence in food and diet and the toxicological implications. Food Additves and Contaminants, **7**:717–768.

Walker W, Harremoes P, Rotmans J Von Krauss M (2003): Defining uncertainty: a conceptual basis for uncertainty management in model-based decision support. Journal of Integrated Assessment: **4 (1)** 5-17

Walker, R. (1995): The conversion of nitrate into nitrite in several animal species and man. In: Health aspects of nitrate and its metabolites (particularly nitrite). Proceedings of an international workshop. Bilthoven (Netherland), 8-10 November 1994, Strasbourg, Council of Europe Press, pp. 115-123.

Wallace, H., Gold, E., & Dooley, S. (1967): Availability and usefulness of selected health and socioeconomic data for community planning. American Journal of Public Health, **57**, 762-771.

Waller K, Swan S, DeLorenze G, Hopkins B (1998): Trihalomethanes in drinking water and spontaneous abortion. Epidemiology 1998; **9**:134-140

Walters, C.L, and Smith, P.L.R (1981): The effect of water-borne nitrate on salivary nitrite. Food chemistry and toxicology; **16**,297-302.

Walter S (1976): The estimation and interpretation of attributable risk in health research. Biometric; **32**, pg829-849.

Walton, G. (1951): Survey of literature relating to infant methaemoglobinaemia due to nitrate-contaminated water. American Journal of Public health; **41**, 986-996.

Wang YL, Lu SX, Li MX (1979): Determination of nitrates and nitrites in well water from Yaocun commune, Linxien County, Henan province (author's transl). Zhonghua Zhong LiuZa Zhi, **1**:201–205

Water Act (2003): www.legislation.gov.uk/ukpga/2003/37/contents

Ward MH, Mark SD, Cantor KP, Weisenburger DD, Correa-Villasenor A, Zahm SH. (1996): Drinking water nitrate and the risk of non-Hodgkin's lymphoma. Epidemiology **7**:465–471.

Ward MH, Pan WH, Cheng YJ et al. (2000): Dietary exposure to nitrite and nitrosamines and risk of nasopharyngeal carcinoma in Taiwan. Int J Cancer, **86**:603–609.

Ward M, Dekok TM, Levallois P,Brender J, Gulis G, Nolan B, VanDerslice J (2005a): Workgroup Report: Drinking water Nitrate and health – Recent Findings and Research Needs. Env. Health Persp. **Vol.113 No.11**, pg 1607-1614.

Ward MH, Heineman EF, McComb RD, Weisenburger DD (2005b): Drinking water and dietary sources of nitrate and nitrite and risk of glioma. J Occup Environ Med, **47**:1260–1267.

Ward MH, Cerhan JR, Colt JS, Hartge P (2006): Risk of non-Hodgkin lymphoma and nitrate and nitrite from drinking water and diet. Epidemiology, **17**:375–382.

Ward M, Kilfoy B, Weyer P, Anderson K, Folsom A, Cerhan J (2010): Nitrate intake and the risk of thyroid cancer and thyroid disease. Epidemiology **21(3):** 389-395: May 2010

Ward MH, Cantor KP, Cerhan J, Lynch CF, Hartge P. (2004). Drinking water nitrate and cancer: results from recent studies in the Midwestern United States. Epidemiology **15**:S214.

Ward MH, Cantor KP, Riley D, Merkle S, Lynch CF. (2003): Nitrate in public water supplies and risk of bladder cancer. Epidemiology **14**:183–190.

Wartenberg D, Simon R (1995): Integrating epidemiologic data into risk assessment. Am. Journal of Public Health; **Vol. 85 No 4**, pg491-3.

Washington State Dept of Health (2002): Drinking water quality: www.doh.wa.gov assessed 18/2/10.

Webb A, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, Ahluwalia A (2008):Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. Hypertension 2008, **51:**784-790.

Weed DL (2002): Environmental epidemiology: basics and proof of cause -and -effect. Toxicology 2002; **181**-182:399-403.

Weed DL (1997). On the use of causal criteria: Int J Epidemiol 1997; **26**:1137 - 41.

Weisburger JH, Marquardt H, Hirota N, Mori H, Williams GM (1980): Induction of cancer of the glandular stomach in rats by an extract of nitrite-treated fish. J Natl Cancer Inst, **64:**163–167.

Weisenburger D (1993): Potential health consequences of ground-water contamination of nitrates in Nebraska. Nebr Med Journal 1993; **78**: 7-10.

Welshons, W. V., Nagel, S. C., Thayer, K. A., Judy, B. M.; vom Saal, F. S (1999): Low-dose bioactivity of xenoestrogens in animals: foetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol. Indust. Health **15**, 12–25

Welshons WV, Nagel SC, vom Saal FS (2006): Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology **147**:S56–S69.

Welshons W, Thayer K, Judy B, Taylor J, Curran E, vom Saal F (2003): Large Effects from Small Exposures. I. Mechanisms for Endocrine-Disrupting Chemicals with Estrogenic Activity. Env. Health Persp: **Vol.111 (8)**, pp994 -1006.

Westin, Jerome B (1990): Ingestion of Carcinogenic N-Nitrosamines by Infants and Children. Archives of Environmental Health, **45:6**. 359-363.

Westrell, T., Andersson, Y. & Stenstrom, T. (2004): Drinking water consumption patterns in Sweden. In: Microbial Risk Assessment and Its Implications for Risk Management in Urban Water Systems (ed. T. Westrell). University of Linkoping, Sweden, Thesis.

Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA,Nadal A, Sonnenschein C, Watson CS, Zoeller RT, Belcher SM (2007): Invitro molecular mechanisms of bisphenol A action. Reprod Toxicol **24**:178–198

Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA (2010): Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999–2004. Environ Health Perspect **118**:825–832

Weyer P (2001): Nitrate in drinking water and human health. University of Illinois Urbana: Campaign Safety and Health Conference 2001.

Weyer PJ, Cerhan JR, Kross BC, Hallberg GR, Kantamneni J, Breuer G, Jones MP, Zheng W, Lynch CF.(2001): Municipal drinking water nitrate level and cancer risk in older women: the Iowa Women's Health Study. Epidemiology 2001; **12(3):** 327-338.

Weyer PJ, Kantamneni JR, Riley DG. (2009): Iowa Statewide Rural Well Water Survey Phase 2 (SWRL2): Results and Analysis. Iowa City, IA: Center for Health Effects of Environmental Contaminants, University of Iowa; 2009.

Wingspread Conference on the Precautionary Principle, January 26, 1998. Wingspread Statement on the Precautionary Principle: Science & Environmental Health Network (Science, Ethics and Action in the Public Interest) http://www.sehn.org/wing.html (accessed December 2, 2014).

Wiklund J, Wertz N, Gorski J (1981): A comparison of oestrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzman rats. Endocrinology **109**:1700–1707.

Wilson (2010): The US Environmental Protection Agency. Office of the Inspector General: Scientific Analysis of Perchlorate. Report No. 10-P-0101. April 19, 2010.

Wilson R, Crouch E (2001): Risk – Benefit. Havard University Press 2001

Wink DA, Kasprzak KS, Maragos CM, Elespuru RK, Misra M, Dunams TM, Cebula TA, Koch WH, Andrews AW, Allen JS (1991): DNA deaminating ability and genotoxicity of nitric oxide and its progenitors. Science **254,** 100-1003.

Wink DA and Mitchell JB (1998): Chemical biology of nitric oxide: insights into regulatory, cytotoxic and cytoprotective mechanisms of nitric oxide. Free Radic Biol Med (1998) **25**: 434-56.

Winterbourn C, McGrath B, Carrel R (1976): Reaction involving superoxide and normal and unstable haemoglobins. Biochem., J (1976) **155**, 493-502.

Winton E, TardiffR, McCabe L (1971): Nitrate in drinking water. J. Amer. Water Works Assn. **63**:95-8.

Wishnok, J.S., Tannenbaum, S.R., Tamir, S. and De Rojas-Walker, T (1995): Endogenous formation of nitrate. In: Health aspects of nitrate and its metabolites (particularly nitrite). Proceedings of an international workshop. Bi1thoven (Netherlands), 8-10 November 1994, Strasbourg, Council of Europe Press, pp.151-179.

Wolff JC (1994): Transport of iodide and other anions in the thyroid. Physiology Reviews 1994: **1**: 45–90.

Wolff J (1998). Perchlorate and the thyroid gland (1998): Pharmacol.Review, 1998; **50**:89–105.

World Bank Group (1998): Comparative Risk Assessment Pollution Prevention and Abatement Handbook pp 45-53.

WHO (1958): International standards for drinking water. Geneva: World health Organisation.

WHO (1978): Nitrates, nitrites and N-nitroso compounds. WHO Env. Health Criteria **5.**

WHO (1985a): Guidelines for the study of dietary intake of chemical contaminants. World Health Organisation (WHO Offset Publication No 87)

WHO (1985b): Health hazards from nitrate in drinking water. Report on a WHO meeting, Copenhagen, 5-9 March 1984. WHO, Regional Office for Europe. Environmental Health Series, No.1.

World Health Organisation (1985c): Health hazards from nitrates in drinking water. WHO, Copenhagen 1985.

WHO (1993a): IPCS: Environmental Health Criteria No. 150. WHO Geneva.

WHO (1993b): Guidelines for drinking water quality. Recommendations: Vol.1. 2nd edition. WHO, Geneva.

WHO (1995): Evaluation of certain food additives and contaminants. Geneva, World Health Organisation, Joint FAO/WHO Expert Committee on Food Additives, Geneva, pp29-35 (WHO Technical Report Series No.859).

WHO (1996a): WHO, Guidelines for drinking water quality. Health Criteria and Other Supporting Information, Vol.2: 2nd edition, WHO Geneva.

WHO (1996b): WHO: Updating and revision of the air quality guidelines for Europe, Report of a WHO Working Group on Volatile Organic Compounds. WHO Regional Office for Europe, 1995.

WHO (1996): Toxicological evaluation of certain food additives and contaminants. Prepared by the Forty-Fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva.

WHO (1996): Guidelines for drinking-water quality. Health Criteria and other supporting information, second edition, Volume 2. World Health Organization, Geneva.

WHO (1999): Principles for the Assessment of Risks to Human Health from Exposure to Chemicals. World Health Organization: Env. Health Criteria 210.

WHO Europe (2002): Water and health in Europe. Regional publications. European series No. 93, 2002

World Health Organisation (2003b): WHO, Environmental burden of disease series, No.1.

WHO (2003a): Assessing microbial safety and drinking water: Improving approaches and methods. IWA Publishing 2003.

WHO (2003b): World Health Organisation. Introduction and methods: Assessing the environmental burden of disease at national and local levels. Geneva, World Health Organization: www.who.int/quantiying_ehimpacts/publications/9241546204/en/index.html, accessed 16/1/2011.

WHO (2004a): Nitrates and Nitrites in Drinking Water. WHO, Drinking Water quality, July 2004.

WHO (2004b): Guidelines for Drinking Water Quality. World Health Organization, Geneva.

WHO (2004c): World Health Organisation global database on iodine deficiency. Geneva, Switzerland: http://www.who.int/vmnis/database/iodine/en/ (accessed May 8, 2014).

World Health Organization (2006): Reducing salt intake in populations. WHO Forum and Technical Meeting, Paris. World Health Organization, Geneva, Switzerland.

WHO/UNICEF/ICCIDD (2007): World Health Organization; United Nations Children's Education Fund; International Council for Control of Iodine Deficiency Disorders. Assessment of Iodine Deficiency Disorders and Monitoring their Elimination: A Guide for Programme Managers; World Health Organization: Geneva, Switzerland, 2007.

World Health Organization (2008): Salt as a vehicle for fortification: report of a WHO Expert Consultation. World Health Organization, Geneva, Switzerland.

WHO (2009): Water Safety Plan Manual: Step- by- step risk management for drinking water suppliers- How to develop and implement a water safety plan. A step-by-step approach using 11 learning modules.

World Health Organisation (2011a): Water and Public Health. WHO Seminar pack for Drinking water Quality. www.who.int/water_sanitation_health/dwq/501.pdf. Assessed 05/09/2011

World Health Organisation (2011b): Guidelines for drinking water Quality: 4th Edition. WHO Publications (2011)

World Health Organisation (2011c): Global health Observatory. Use of improved drinking water sources. www.who.int/gho/mdg/environmental_sustainability/water_text/en/index.html. Accessed 05/09/2011.

WHO/PAHO (2011d): Regional Expert Group for Cardiovascular Disease Prevention through Population-Wide Dietary Salt Reduction 2011 Final report. http://new.paho.org/hq/index.php?option=com_content&view=article&id=2015&Itemid=1757&lang=en (accessed May 3, 2014).

Woodruff TJ, Zota AR, Schwartz JM (2011): Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environ Health Perspect. **119**:878–885.

World Bank (1985): Population Health and nutrition Department: Bangladesh: Food and nutrition sector review mission: Cost effectiveness of food and nutrition intervention programs. Washington, DC. World Bank, 1985 (No.4974-BD).

Wolff J (2001): Physiology and pharmacology of iodized oil in goiter prophylaxis. Medicine, 2001, **80**:20–36.

Wozniak AL, Bulayeva NN, Watson CS (2005): Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor- mediated Ca2 fluxes and prolactin release in GH3/B6 pituitary tumor cells. Environ Health Perspect **113:**431–439.

Wright J; Cave B (2013): Assessing health needs. In: Oxford book of public health practice pg38-49. Guest C, Ricciardi W, Kawachi I, Lang I (Eds). Oxford University Press, 2001.

Wyngaarden JB, Wright BW, Ways P (1952): The effect of certain anions upon the accumulation and retention of iodine by thyroid gland. Endocrinology, 1952; 50:537–541.

Wjngaarden J, Stanbury J B, Rapp B (1953): The effect of iodine, perchlorate, thiocynate and nitrate administration upon iodine-concentrating mechanism of the rat thyroid. Endocrinology **52**, 568-574.

Xiang YY, Wang DY, Tanaka M et al. (1995): Efficient and specific induction of oesophageal tumours in rats by precursors of N-nitrososarcosine ethyl ester. Pathology International, **45**:415–421.

Ximenes, M.I.N., Rath, S. and Reyes, F.G.R. (2000): Polargraphic determination of nitrate in vegetables. Talanta, **51**, 49-56.

Xu G, Song P, Reed PI (1992): The relationship between gastric mucosal changes and nitrate intake via drinking water in a high-risk population for gastric cancer in Moping County, China. Eur J Cancer Prev **1(6):**437–443. 1992.

Yang CY, Cheng MF, Tsai SS, Hsieh YL (1998): Calcium, magnesium, and nitrate in drinking water and gastric cancer mortality. Japan J Cancer Res **89(2):**124–130. 1998.

Yang Y, Lerner D.N, Barrett M. H, Tellam J.H (1999): Quantification of groundwater recharge in the city of Nottingham UK. Environmental Geology 1999

Yang C, Tsai S, Chiu H (2009): Nitrate in drinking water and risk of death from pancreatic cancer in Taiwan. Journal of Toxicology and Environmental Health, Part A, **72(6)**397-401.

Yang C, Wu D, Chang C (2007): Nitrate in drinking water and risk of death from colon cancer in Taiwan. Environ. International Vol. **33, Issue 5** pg 649-653

Yano SS, Danish EH, Hsia YE (1982): Transient methaemoglobinemia with acidosis in infants. J Pediatrics **100**:415–418.

Yielding K (1993): Primary and secondary risk factors for birth defects. Env.Health Persps. supplements Vol. **101 (suppl.3**): 285-290.

Ying L, Hofseth LJ (2007): An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. Cancer Research 2007, **67**:1407-1410.

Yocom JE (1992): Indoor/outdoor air quality relationship: a critical review. Journal of the Air Pollution Control Association **32**, 500-606.

Yoshida A, Harada T, Maita K (1993): Tumor induction by concurrent oral administration of ethylenethiourea and sodium nitrite in mice. Toxicol Pathol, **21**:303–310.

Yoshida Y, Hirose M, Takaba K, Kimura J, Ito N (1994): Induction and promotion of forestomach tumors by sodium nitrite in combination with ascorbic acid or sodium ascorbate in rats with or without Nmethyl- N′-nitro-N-nitrosoguanidine pre-treatment. Int J Cancer, **56**:124–128.

Young CP, Morgan-Jones M (1980): A hydrochemical survey of the chalk groundwater of the Banstead, Surrey, with particular reference to nitrates. Journal of Institute of Water Engineers and Scientists **34**, 213-236

Zaki A, Chaoui A, TalibiA, Derouche F, AboussaouiraK, Zarrouck A, Cait A, Himmi T (2004): Impact of nitrate intake in drinking water on thyroid gland activity of male rats. Toxicology Letters: **Vol.147, issue1**, pp27-33.

Zeger SL, Thomas D, Dominici F, Samet JM, Schwartz J, Dockery D, Cohen A (2000): Exposure measurement error in time-series studies of air pollution: concepts and consequences. Environ Health Perspective 2000 May; 108(5):419-26.

Zeeb H (2011): Indoor radon and lung cancer. In: Environmental burden of disease associated with inadequate housing. Methods for quantifying health impacts of selected housing risks in the WHO European Region. Pg. 113-123. Edited by: Braubach, M., Jacobs, D.E, Ormandy, D.

Zender R, Bachand AM, Reif JS (2001): Exposure to tap water during pregnancy. J Expo Anal Environ Epidemiol. **11(3)**:224–230.

Zhang W.L, Tian Z.X; Li X.Q (1996): Nitrate pollution of groundwater in Northern China. Agriculture, Ecosystems and Environment, **59**, 223-31.

Zhao ZG, Guo XG, Ba CX, Wang W, Yang YY, Wang J, Cao HY (2012): Overweight, obesity and thyroid cancer risk: a meta-analysis of cohort studies. J. Int Med Res 2012; **40(6):**2041-50.

Zimmermann M (2008): Iodine requirements and the risks and benefits of correcting iodine deficiency in populations. Journal of Trace Elements in Medicine and Biology, 81-92.

Zimmermann M. B (2009a): Iodine deficiency. Endocrine Review; **30:** 376–408.

Zimmermann, M.B (2009b): Iodine deficiency in pregnancy and the effects of maternal iodine supplementation on the offspring: A review. American Journal of Clinical Nutrition, **89,** 668S–672S.

Zimmerman MB, Andersson M (2010): Prevalence of iodine deficiency in Europe in 2010. Annals of Endocrinology; **72(2)**:164-6.

Zimmermann MB (2011): Iodine deficiency in industrialized countries. Clinical Endocrinology 2011; **75**:287–88.

Zimmermann, M.B., Andersson, M (2012a): Update on iodine status worldwide. Curr.Opin.Endocrinol. Diabetes Obes.2012, **19**, 382–387.

Zimmermann, M.B.; Andersson, M (2012b): Assessment of iodine nutrition in populations: Past, present, and future. Nutrition Reviews. 2012, **70**, 553–570.

Zimmermann, M.B (2012): The effects of iodine deficiency in pregnancy and infancy. Paediatrics Perinatal Epidemiology, **26**, 108–117.

Zoeller, R.T. and Crofton, K.M. (2005): Mode of action: Developmental thyroid hormone insufficiency- neurological abnormalities resulting from exposure to propylthiouracil. Critical Reviews Toxicology, **35(8–9):** 771–781.

Zweier JL, Wang P, Kuppusamy P (1995): Direct measurement of NO generation in the ischemic heart using electron paramagnetic resonance spectroscopy. Journal of Biological Chemistry **270:** 304-7.

Appendix 1: Database Search Terms

PMC ("nitrates"[MeSH Terms] OR "nitrates"[All Fields] OR "nitrate"[A

Search Details

Query Translation:

```
("nitrates"[MeSH Terms] OR "nitrates"[All Fields]
OR "nitrate"[All Fields]) AND ("drinking water"[MeSH Terms]
OR ("drinking"[All Fields] AND "water"[All Fields])
OR "drinking water"[All Fields]) AND ("thyroid
diseases"[MeSH Terms] OR ("thyroid"[All Fields]
AND "diseases"[All Fields]) OR "thyroid diseases"[All
Fields] OR ("thyroid"[All Fields] AND "disorders"[All
Fields]) OR "thyroid disorders"[All Fields])
```

Search URL

Result:

349

Database:

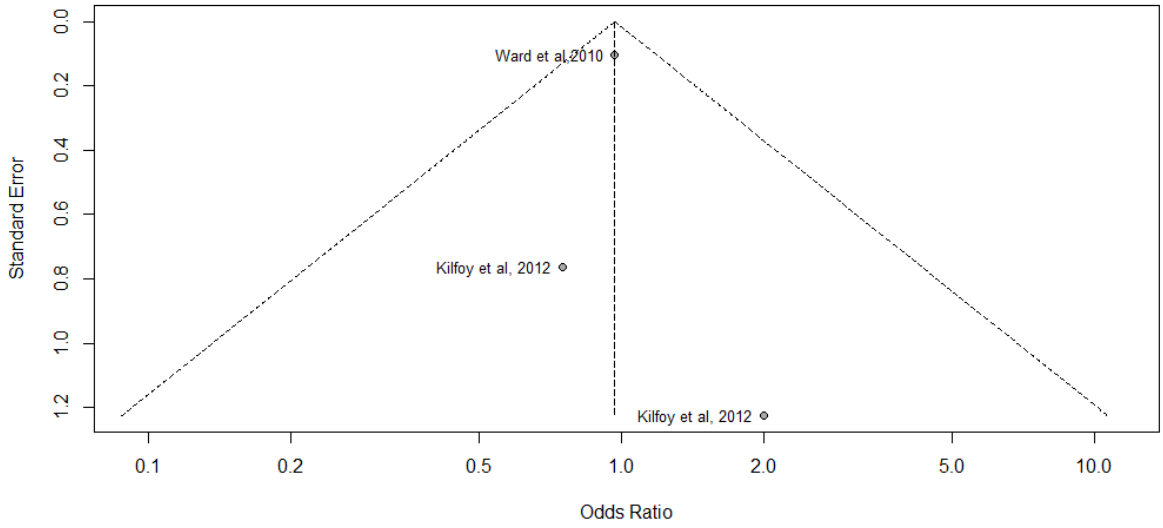
PMC

User query:

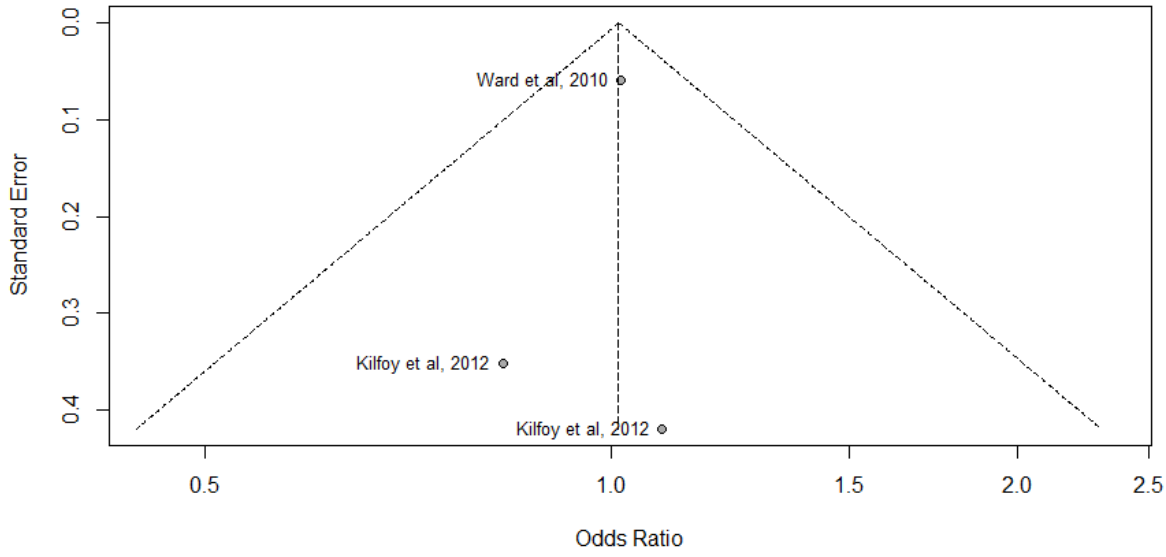
("nitrates"[MeSH Terms] OR "nitrates"[All Fields] OR "nitrate"[All Fields]) AND ("drinking water"[MeSH Terms] OR ("drinking"[All Fields] AND "water"[All Fields]) OR "drinking water"[All Fields]) AND ("thyroid diseases"[MeSH Terms] OR ("thyroid"[All Fields] AND "diseases"[All Fields]) OR "thyroid diseases"[All Fields] OR ("thyroid"[All Fields] AND "disorders"[All Fields]) OR "thyroid disorders"[All Fields])

Appendix 2: Funnel Plots

Clinical hyperthyroidism

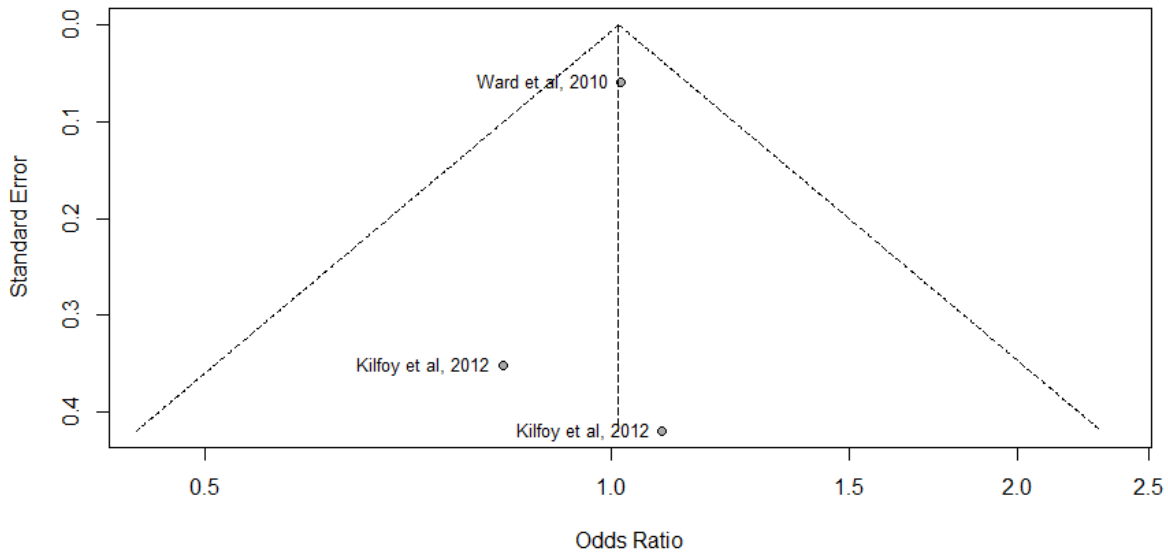


Subclinical hyperthyroidism

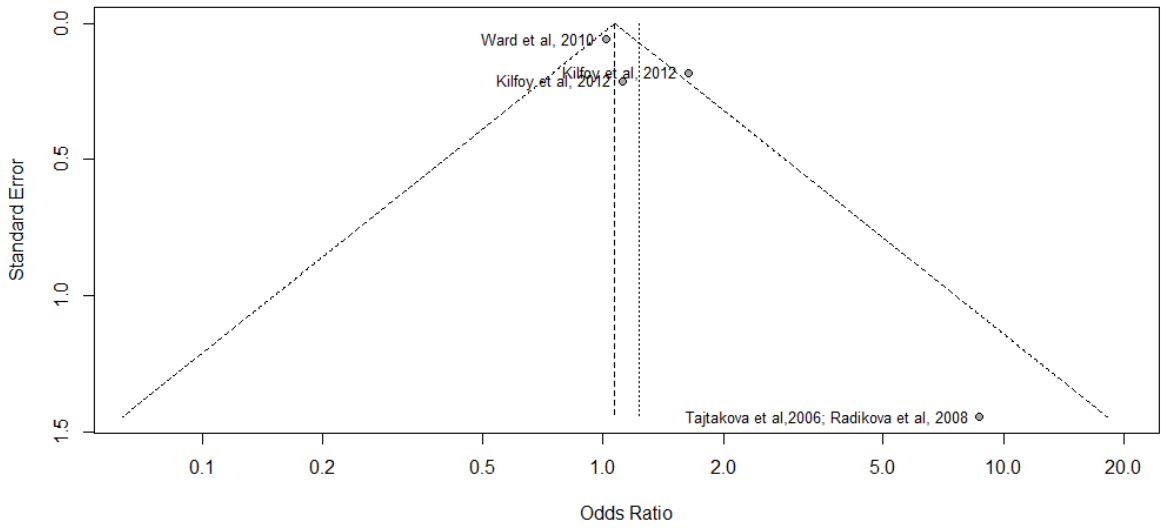


Appendix 2 (Continued): Funnel Plots

Clinical hypothyroidism

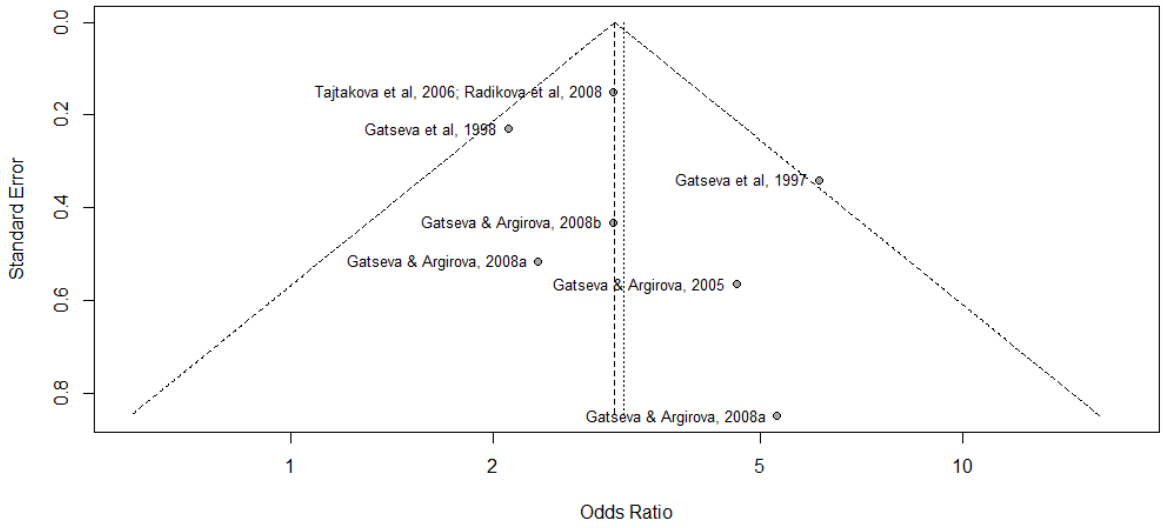


Funnel plot subclinical hypothyroidism



Appendix 2 (Continued): Funnel Plots

Funnel Plot - Goitre



APPENDIX 3: Letter to Water Companies requesting nitrate data in public water.

Suffolk Coastal District Council

Melton Hill, Woodbridge, Suffolk IP12 1AU

Tel: (01394) 383789

Fax: (01394) 385100

Minicom: (01394) 444211

DX: Woodbridge 41400

Website: www.suffolkcoastal.gov.uk

Please ask for: G.N.Onuoha

Direct Dial:01394 444267

Email: George.onuoha@eastsuffolk.gov.uk

Our Ref:

Your Ref:

The Water Quality Manager

(Water Company Name)

1 August 2010

Dear Sir/Madam

WATER SUPPLY (WATER QUALITY) REGULATIONS 2000: PERMISSION TO USE NITRATE DATA FROM YOUR WATER COMPANY FOR MY PhD RESEARCH PROJECT.

I work for Suffolk Coastal District Council (Environmental Health Section) and I am currently undertaking a study on the quantitative risk assessment of exposure to nitrates in drinking water and adverse human health outcomes in East Anglia. This study is being undertaken at the School of Health & Human Sciences, University of Essex as part of my PhD.

I am at the data collection stage of the research project and I am to obtain information from Water Companies in East Anglia on nitrate levels in drinking water sources.

I would be most grateful if you could assist me with information on nitrate levels in your water zone for the last 10 years. The following information is required; water source type (e.g. groundwater, surface water etc); number of samples taken each year, raw nitrate levels before blending (minimum and maximum levels is acceptable) and the postcode of the areas supplied.

Please note that any data supplied will be taken as consent to use your data for the purposes of this research project only, and be assured that any information provided will be treated with absolute confidentiality and anonymity such that no particular individual or Water Company will be identified.

Thank you very much for your time and I look forward to hearing from you.

Yours faithfully, Mr George Onuoha

Appendix 4: Letter and Questionnaire to Local Authority Environmental Health

Suffolk Coastal District Council

Melton Hill, Woodbridge, Suffolk IP12 1AU

Tel: (01394) 383789

Fax: (01394) 385100

Minicom: (01394) 444211

DX: Woodbridge 41400

Website: www.suffolkcoastal.gov.uk

Please ask for: G.N.Onuoha

Direct Dial:01394 444267

Email: environment@eastsuffolk.gov.uk

Head of Environmental Health

(Local Authority Name)

Our Ref:

Your Ref:

1 August 2010

Dear Sir/Madam

PRIVATE WATER SUPPLIES: PERMISSION TO USE NITRATE DATA FROM YOUR LOCAL AUTHORITY FOR MY PhD RESEARCH PROJECT

I work for Suffolk Coastal District Council (Environmental Health Team) and I am currently undertaking a study on the quantitative risk assessment of exposure to nitrates in drinking water and adverse human health outcomes in East Anglia. This study is being undertaken at the School of Health & Human Sciences, University of Essex as part of my PhD. I am at the data collection stage of the research project and I am to obtain information from local authorities in East Anglia on the level of nitrates in PWS in their areas.

I would be most grateful if you could spare a few minutes of your time to assist me with this data collection exercise by completing the attached questionnaire. The questionnaire is very brief and I have enclosed a self-addressed envelope for its return at your earliest convenience.

Please note that by completing this questionnaire, you give consent to use data for the purposes of this research project only, and be assured that any information provided will be treated with absolute confidentiality and anonymity such that no particular individual/s or local authority will be identified.

Thank you very much for your time and I look forward to hearing from you.

Yours faithfully, Mr George Onuoha

Encs

Appendix 4(continued): Letter and Questionnaire to Local Authority Environmental Health.

NITRATES QUESTIONNAIRE

1. Do you have any private water supplies (PWS) in your authority?

   YES (If yes, please answer questions 2-4) NO (If No, please return questionnaire in the SAE)

2. How many PWS are there in your authority? (an estimate will be acceptable if the actual number is not readily available)

3. Are there any PWS in your area with nitrate levels above the standard set in Schedule 1 of the Private Water Supplies Regulations 2009 - (50mg/l)?

   Yes No

4. What are the levels of nitrates in PWS in your authority? (Please state the number of samples in each category).

Level (mg/l)	Number
<50	
50-100	
101-200	
201-300	
>300	

CONSENT FORM

I give consent to use this data for the purposes of this research project only.

Signed……..Date…….

Local authority Name:

Tel No:

Email:

Would you like to know the outcome of the study when finished?

Yes No

Thank you very much for your time.

George Onuoha

Email:gnonuo@essex.ac.uk

Tel. 079 3285 8382

Appendix 5 (Table 3): Nitrate levels in public and private water supplies and the approximate population served in East Anglia in 2001-2010

Nitrate levels (mg/l)	Mean level (mg/l)	Population (P)		
		Mains	PWS	Total (P)
0-5	2.5	677, 884	2754	680,638
6-10	8	140, 586	1050	141,636
11-15	13	516, 772	774	517,546
16-20	18	149, 432	1146	150,578
21-25	23	149, 848	606	150,454
26-30	28	301, 299	546	301,845
31-35	33	366, 586	372	367,132
36-40	38	464, 380	324	464,704
41-45	43	47, 121	552	47,673
46-50	48	22, 500	1074	23,574
51-55	53	-	540	540
56-60	58	-	336	336
61-65	63	-	330	330
66-70	68	-	210	210
71-75	73	-	108	108
76-80	78	-	246	246
81-85	83	-	174	174
86-90	88	-	138	138
91-95	93	-	174	174
96-100	98	-	312	312
101-105	103	-	354	354
106-110	108	-	258	258
111-115	113	-	54	54

Appendix 5; Table 3 (continued): Nitrate levels in public and private water supplies and the approximate population served in East Anglia in 2001-2010.

Nitrate levels (mg/l)	Mean level (mg/l)	Population (P)		
		Mains	PWS	Total (P)
116-120	118	-	114	114
121-125	123	-	42	42
126-130	128	-	66	66
131-135	133	-	42	42
136-140	138	-	72	72
141-145	143	-	54	54
146-150	148	-	102	102
151-155	153	-	18	18
156-160	158	-	42	42
161-165	163	-	12	12
166-170	168	-	18	18
171-175	173	-	-	-
176-180	178	-	30	30
181-185	183	-	6	6
186-190	188	-	30	30
191-195	193	-	-	-
196-200	198	-	108	108
201-205	203	-	36	36
206-210	208	-	54	54
211-215	213	-	66	66
216-220	218	-	6	6

Appendix 5, Table 3(continued): Nitrate levels in public and private water supplies and the approximate population served in East Anglia in 2001-2010.

Nitrate levels (mg/l)	Mean level (mg/l)	Population (P)		
		Mains	PWS	Total (P)
221-225	223	-	-	-
226-230	228	-	30	30
231-235	233	-	6	6
236-240	238	-	24	24
241-245	243	-	-	-
246-250	248	-	6	6
276-280	278	-	12	12
281-285	283	-	-	-
286-290	288	-	12	12
316-320	318	-	6	6
376-380	378	-	6	6
386-390	388	-	18	18
416-420	418	-	24	24
431-435	433	-	6	6
466-470	468	-	6	6
Total		**2,836,408**	**13,510**	**2,849,918**

APPENDIX 6: LETTER TO RESIDENTS AND QUESTIONNAIRE

Suffolk Coastal District Council

Melton Hill, Woodbridge, Suffolk IP12 1AU

Tel: (01394) 383789

Fax: (01394) 385100

Minicom: (01394) 444211

DX: Woodbridge 41400

Website: www.suffolkcoastal.gov.uk

1 August 2010

(Resident Address)

Dear Resident

You are being invited to take part in a research study on the Quantitative risk assessment of exposure to nitrates in drinking water and adverse human health outcomes by completing the attached questionnaire. This study is being undertaken at the School of Health & Human Sciences, University of Essex as part of my PhD. Before you decide whether or not to take part, please take time to read the following information. It explains why and how the research is being done.

Aim of the study

Currently the only known human health effect associated with consumption of nitrates in drinking water is infantile methaemoglobinemia (Blue-baby syndrome) and the current drinking water standard of 50mg/l has been set to protect infants against this disease. However, the appropriateness of this standard in protecting against other potential health outcomes and the magnitude of risk (if any) posed by this drinking water contaminant has not been previously evaluated. This study therefore proposes to determine whether there is any health risk (apart from blue–baby syndrome) associated with exposure to nitrates in drinking water, and to quantify the risk (if any).

Questionnaire

The questionnaire is very short and should take about 5 minutes to complete. It seeks to gather information on the amount of tap-water intake among residents in Suffolk Coastal District Council. The questionnaire does not require any personal details. All data will be stored, analysed and reported in compliance with the Data Protection Act 1998.

If you decide to participate, please complete and return the questionnaire in the envelope provided (no stamp is required). Please note that return of the completed questionnaire will be taken as your consent to use the information provided for the purposes of this study only.

If you do not wish to take part in this study, please tick the box below and return the questionnaire in the envelope provided.

☐

Should you wish to discuss this or require further information, please do not hesitate to contact me at the above address or with the following email address: gnonuo@essex.ac.uk

Thank you very much for your time.

George Onuoha

PhD Candidate in Public Health, University of Essex

Questionnaire on the amount of tap-water intake by residents in Suffolk Coastal District Council.

1. Age

Person 1	Person 2	Person 3	Person 4	Person 5	Person 6

2. Sex

Person 1	Person 2	Person 3	Person 4	Person 5	Person 6

3. What is the source of the water supply to your home?(Please tick one box)

a) Private well/borehole ☐

b) Public (mains) water ☐

APPENDIX 6 (continued): LETTER TO RESIDENTS AND QUESTIONNAIRE

4. On average, how much tap-water do you drink each day? (as we only need an estimate, please write down the number of standard size glasses or mugs drank each day, including for hot and cold drinks)

Person 1	**Person 2**	**Person 3**	**Person 4**	**Person 5**	**Person 6**

5. On average, how much bottled water do you drink each day? (including for cold and hot drinks)

Person 1	Person 2	Person 3	Person 4	Person 5	Person 6

CONSENT FORM

1. I confirm that I have read and understand the invitation letter dated 1 August, 2010, for this study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.
3. I understand that my responses will be recorded and may be analysed by responsible individuals from the University of Essex for the purposes of this research project only. I give permission for these individuals to have access to my recorded responses.
4. I give consent to use this data for the purposes of this research project.

Signed…… Date……

Thank you very much for your time.

George Onuoha

Email: gnonuo@essex.ac.uk

APPENDIX 7: ETHICAL APPROVAL

University of Essex

School of Health and Human Sciences
Telephone: 01206 872854
Fax: 01206 873765
E-mail: hhs@essex.ac.uk

Colchester Campus
Wivenhoe Park
Colchester CO4 3SQ
United Kingdom
Telephone: 01206 873333
Fax: 01206 873598
Web: www.essex.ac.uk

03 September 2010

MR G.N. ONUOHA
52 SALLOWS CLOSE
IPSWICH
SUFFOLK
IP1 4BJ

Dear George,

Re: Ethical Approval Application (Ref 996)

Further to your application for ethical approval, please find enclosed a copy of your application which has now been approved by the Research Director.

Kind regards,

Mel Hassack
Graduate Administrator
Health and Human Sciences

cc. Prof. Ian Colbeck, Supervisor
Sarah Manning-Press, REO

THE QUEEN'S AWARDS FOR ENTERPRISE 2005

Appendix 8: Health Protection Agency (Public Health England) Advice Note on Nitrates in drinking water.

East of England

Health Protection Agency

Your Ref:
Our Ref: Nit.Commerc.priv.50-100.Feb05

Norfolk, Suffolk & Cambridgeshire
Health Protection Unit

Suffolk Health Protection Team
PO Box 170
South Building
St Clements
Foxhall Road
Ipswich
Suffolk IP3 8LS

Tel: +44 (0)1473 329583
Fax: +44 (0)1473 329090
E-mail: torbjorn.sundkvist@lhp.nhs.uk
www.hpa.org.uk

Dear

RE: ELEVATED NITRATE LEVELS IN A COMMERCIAL PRIVATE WATER SUPPLY SERVING:

Samples collected from the above commercial private water supply on revealed nitrate levels of mg per litre.

Levels of nitrate between 50 and 100 mg per litre pose a potential risk for unborn babies and babies under six months of age as their systems are not yet mature enough to handle it.

We would, therefore, strongly recommend that in premises where there could be pregnant consumers or babies under six months of age, the following advice is followed:

- Use low-nitrate bottled water for all circumstances where water is consumed. All bottled waters have a list of their constituents, including nitrate, on the bottle and all those commonly offered for sale have very low nitrate levels.
- Please be aware that, due to the high salt content of some bottled waters, their use is **not recommended for babies under twelve months of age**. If in doubt, consumers should consult their GP or other healthcare professional.
- Alternatively, tap water may be blended with low-nitrate bottled water to bring the overall nitrate level of the water consumed to comfortably below 50 mg/l. For instance, if the nitrate in the supply is 80 mg per litre, for all water consumed use half tap water and half low-nitrate bottled water. This will reduce the nitrate level in the water consumed to around 40 mg per litre, which is below the standards in the Private Water supplies Regulations 1991.

Levels of nitrate between 50 and 100 mg per litre only pose a potential health problem for unborn babies and babies under six months of age when the water is consumed regularly.

Yours sincerely

Dr Torbjorn Sundkvist
Consultant in Communicable Disease Control

Appendix 9: Table on Excess Risk of Thyroid Cancer by Age groups in East Anglia

Nitrate levels (mg/l)	**Mean level (C)**	Infant ≤**1yr**			Children **1-6yrs**			Children **7-12yrs**		
		CDI	**RR= mx+1**	**AR** $\frac{RR-1}{RR}$	**CDI**	**RR= mx+1**	**AR** $\frac{RR-1}{RR}$	**CDI**	**RR= mx+1**	**AR** $\frac{RR-1}{RR}$
0-5	2.5	0.25	1.01	$9.9x\ 10^{-3}$	0.20	1.01	$9.9x10^{-3}$	0.09	1.00	0
6-10	8	0.80	1.03	0.03	0.56	1.02	0.02	0.30	1.01	$9.9x10^{-3}$
11-15	13	1.30	1.04	0.04	0.91	1.03	0.03	0.46	1.01	$9.9x10^{-3}$
16-20	18	1.80	1.06	0.06	1.26	1.04	0.04	0.64	1.02	0.02
21-25	23	1.90	1.06	0.06	1.61	1.05	0.05	0.82	1.03	0.03
26-30	28	2.80	1.10	0.09	1.96	1.07	0.06	1.00	1.03	0.04
31-35	33	3.30	1.11	0.10	2.31	1.08	0.07	1.18	0.04	0.05
36-40	38	3.80	1.13	0.11	2.66	1.09	0.08	1.36	1.05	0.05
41-45	43	4.30	1.15	0.13	3.01	1.10	0.09	1.53	1.05	0.06
46-50	48	4.80	1.17	0.14	3.36	1.12	0.11	1.71	1.06	0.06
51-55	53	5.30	1.18	0.15	3.71	1.13	0.11	1.90	1.06	0.06
56-60	58	5.80	1.20	0.17	4.06	1.14	0.12	2.07	1.07	0.07
61-65	63	6.30	1.22	0.18	4.41	1.15	0.13	2.25	1.08	0.07
66-70	68	6.80	1.23	0.19	4.76	1.16	0.14	2.43	1.08	0.08
71-75	73	7.30	1.25	0.20	5.11	1.18	0.15	2.61	1.09	0.09
76-80	78	7.80	1.27	0.21	5.46	1.19	0.16	2.78	1.10	0.09
81-85	83	8.30	1.29	0.22	5.81	1.20	0.17	2.96	1.10	0.10
86-90	88	8.80	1.30	0.23	6.16	1.21	0.17	3.14	1.11	0.10
91-95	93	9.30	1.32	0.24	6.51	1.22	0.18	3.32	1.11	0.11
96-100	98	9.80	1.34	0.25	6.86	1.24	0.19	3.50	1.12	0.11
101-105	103	10.30	1.36	0.26	7.20	1.25	0.20	3.68	1.13	0.11
106-110	108	10.80	.37	0.27	7.56	1.26	0.21	3.86	1.13	0.12
111-115	113	11.30	1.39	0.28	7.91	1.27	0.21	4.03	1.14	0.13
116-120	118	11.80	1.41	0.29	8.26	1.29	0.22	4.21	1.15	0.13
121-125	123	12.30	143	0.30	8.61	1.30	0.23	4.39	1.15	0.14
126-130	128	12.80	1.44	0.30	8.96	1.31	0.24	4.57	1.16	0.14
131-135	133	13.30	1.46	0.31	9.31	1.32	0.24	4.75	1.16	0.14
136-140	138	13.80	1.48	0.32	9.66	1.33	0.25	5.00	1.17	0.15
141-145	143	14.30	1.50	0.33	10.01	1.35	0.26	5.11	1.18	0.15
146-150	148	14.80	1.51	0.34	10.36	1.36	0.26	5.28	1.18	0.16
151-155	153	15.30	1.53	0.35	10.71	1.37	0.27	5.46	1.19	0.16
156-160	158	15.80	1.55	0.35	11.06	1.38	0.27	5.64	1.19	0.16

Appendix 9(continued): Table on Excess Risk of Thyroid Cancer by Age groups in East Anglia.

Nitrate levels (mg/l)	Mean level (C)	Infant ≤1yr			Children 1-6yrs			Children 7-12yrs		
		CDI	RR= mx+1	AR= $\frac{RR-1}{RR}$	CDI	RR= mx+1	AR= $\frac{RR-1}{RR}$	CDI	RR= mx+1	AR= $\frac{RR-1}{RR}$
161-165	163	16.30	1.56	0.36	11.41	1.39	0.28	5.82	1.20	0.17
166-170	168	16.80	1.58	0.37	11.76	1.41	0.29	6.00	1.21	0.17
171-175	173	-	-	-	-	-	-	-	-	-
176-180	178	17.80	1.62	0.38	12.46	1.43	0.30	6.36	1.22	0.18
181-185	183	18.30	1.63	0.39	12.81	1.44	0.30	6.53	1.23	0.19
186-190	188	18.80	1.65	0.39	13.16	1.46	0.31	6.71	1.23	0.19
191-195	193	-	-	-	-	-	-	-	-	-
196-200	198	19.80	1.69	0.41	13.86	1.48	0.32	7.07	1.24	0.19
201-205	203	20.30	1.70	0.41	14.21	1.49	0.33	7.25	1.25	0.20
206-210	208	20.80	1.72	0.41	14.56	1.50	0.33	7.43	1.26	0.21
211-215	213	21.30	1.74	0.42	14.91	1.52	0.33	7.61	1.26	0.21
216-220	218	21.80	1.76	0.43	15.26	1.53	0.34	7.78	1.27	0.21
221-225	223	-	-	-	-	-		-	-	-
226-230	228	22.80	1.79	0.44	15.96	1.55	0.35	8.14	1.28	0.22
231-235	233	23.30	1.81	0.45	16.31	1.56	0.35	8.15	1.28	0.22
236-240	238	23.80	1.82	0.45	16.60	1.58	0.36	8.50	1.29	0.22
241-245	243	-	-	-	-	-		-	-	-
246-250	248	24.80	1.86	0.46	17.36	1.60	0.37	8.86	1.31	0.24
276-280	278	27.80	1.96	0.49	19.46	1.67	0.40	10.00	1.35	0.26
281-285	283	-	-	-	-	-	-	-	-	-
286-290	288	28.80	2.00	0.50	20.16	1.70	0.41	10.29	1.36	0.26
316-320	318	31.80	2.10	0.52	22.26	1.77	0.43	11.36	1.39	0.28
376-380	378	37.80	2.31	0.57	26.46	1.92	0.48	13.50	1.47	0.32
386-390	388	38.80	2.35	0.57	27.16	1.94	0.48	13.86	1.48	0.32
416-420	418	41.80	2.45	0.59	29.26	2.01	0.50	14.93	1.52	0.34
431-435	433	43.30	2.50	0.60	30.31	2.05	0.51	15.46	1.54	0.35
466-470	468	46.80	2.62	0.62	32.76	2.14	0.53	16.71	1.58	0.37

Appendix 9: Table on Excess Risk of Thyroid Cancer by Age groups in East Anglia

Nitrate level (mg/l)	**Mean level (C)**	Adolescents **(13-18yrs)**			Adults (**19-70yrs)**		
		CDI	**RR= mx+1**	**AR=** $\frac{RR-1}{RR}$	**CDI**	**RR= mx+1**	**AR=** $\frac{RR-1}{RR}$
0-5	2.5	0.04	1.00	0	0.05	1.00	0
6-10	8	0.12	1.00	0	0.15	1.00	0
11-15	13	0.19	1.01	$9.9x10^{-3}$	0.24	1.01	$9.9x10^{-3}$
16-20	18	0.27	1.01	$9.9x10^{-3}$	0.33	1.01	$9.9x10^{-3}$
21-25	23	0.35	1.01	$9.9x10^{-3}$	0.42	1.01	$9.9x10^{-3}$
26-30	28	0.40	1.01	$9.9x10^{-3}$	0.52	1.01	0.02
31-35	33	0.50	1.02	0.20	0.61	1.02	0.02
36-40	38	0.60	1.02	0.02	0.70	1.02	0.02
41-45	43	0.65	1.02	0.02	0.79	1.02	0.03
46-50	48	0.72	102	0.03	0.89	1.03	0.03
51-55	53	0.80	1.03	0.03	0.97	1.03	0.03
56-60	58	0.87	1.03	0.03	1.10	1.03	0.04
61-65	63	0.95	1.03	0.04	1.20	1.04	0.04
66-70	68	1.03	1.03	0.04	1.25	1.04	0.04
71-75	73	1.10	1.04	0.04	1.35	1.04	0.05
76-80	78	1.18	1.04	0.05	1.44	1.05	0.05
81-85	83	1.25	1.04	0.05	1.50	1.05	0.05
86-90	88	1.33	1.05	0.05	1.62	1.05	0.06
91-95	93	1.40	1.05	0.05	1.70	1.06	0.06
96-100	98	1.48	1.05	0.06	1.80	1.06	0.06
101-105	103	1.55	1.05	0.06	1.90	1.06	0.06
106-110	108	1.63	1.06	0.06	1.99	1.06	0.07
111-115	113	1.70	1.06	0.06	2.10	1.07	0.07
116-120	118	1.78	1.06	0.06	2.17	1.07	0.07
121-125	123	1.86	1.06	0.06	2.30	1.08	0.08
126-130	128	1.93	1.06	0.06	2.36	1.08	0.08
131-135	133	2.00	1.06	0.06	2.45	1.08	0.08
136-140	138	2.08	10.7	0.06	2.50	1.09	0.08
141-145	143	2.15	1.07	0.06	2.60	1.09	0.08
146-150	148	2.23	1.08	0.07	2.70	1.09	0.08

Nitrate level (mg/l)	**Mean level (C)**	**Adolescents (13-18yrs)**			**Adults (19-70yrs)**		
		CDI	**RR= mx+1**	**AR=** $\frac{RR-1}{RR}$	**CDI**	**RR= mx+1**	**AR=** $\frac{RR-1}{RR}$
151-155	153	2.31	1.08	0.07	2.80	1.10	0.09
156-160	158	2.38	1.08	0.07	2.90	1.10	0.09
161-165	163	2.46	1.08	0.07	3.00	1.10	0.09
166-170	168	2.53	1.09	0.08	3.10	1.11	0.10
171-175	173	-	-	-	-	-	-
176-180	178	2.70	1.09	0.08	3.30	1.11	0.10
181-185	183	2.76	1.09	0.08	3.40	1.12	0.11
186-190	188	2.84	1.10	0.09	3.50	1.12	0.11
191-195	193	-	-	-	-	-	-
196-200	198	3.00	1.10	0.09	3.65	1.13	0.11
201-205	203	3.06	1.11	0.10	3.70	1.13	0.11
206-210	208	3.14	1.11	0.10	3.80	1.13	0.11
211-215	213	3.21	1.11	0.10	3.90	1.13	0.11
216-220	218	3.30	1.11	0.10	4.00	1.14	0.12
221-225	223	-	-	-	-	-	-
226-230	228	3.44	1.12	0.10	4.20	1.14	0.12
231-235	233	3.52	1.12	0.11	4.30	1.15	0.13
236-240	238	3.59	1.12	0.11	4.40	1.15	0.13
241-245	243	-	-	-	-	-	-
246-250	248	3.74	1.13	0.11	4.60	1.16	0.14
276-280	278	4.20	1.14	0.11	5.10	1.18	0.15
281-285	283	-	-	-	-	-	-
286-290	288	4.45	1.15	0.13	5.30	1.18	0.15
316-320	318	4.80	1.17	0.14	5.90	1.20	0.17
376-380	378	5.71	1.20	0.17	6.97	1.24	0.19
386-390	388	5.86	1.20	0.17	7.20	1.25	0.20

Appendix 9 (continued): **Table on Excess Risk of Thyroid Cancer by Age groups in East Anglia.**

Nitrate level (mg/l)	**Mean level (C)**	Adolescents **(13-18yrs)**			Adults (**19-70yrs)**		
		CDI	**RR= mx+1**	**AR=** $\frac{RR-1}{RR}$	**CDI**	**RR= mx+1**	**AR** $\frac{RR-1}{RR}$
416-420	418	6.31	1.22	0.18	7.70	1.27	0.21
431-435	433	6.53	1.23	0.19	8.00	1.27	0.21
466-470	468	7.06	1.24	0.19	8.64	1.30	0.23

Abbreviation:
CDI = chronic daily intake.
RR = relative risk.
AF = attributable fraction.
PAF = population attributable fraction.
Mg/kgbw/day = miligram per kilogram bodyweight per day.