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RISKS AND BENEFITS OF DRINKING WATER TREATMENT—FOCUSING ON CHILD HEALTH AND PRENATAL DEVELOPMENT

Melle Säve-Söderbergh

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RISKS AND BENEFITS OF DRINKING WATER TREATMENT—FOCUSING ON CHILD HEALTH AND PRENATAL DEVELOPMENT

THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

Melle Säve-Söderbergh

Principal Supervisor:
Professor Agneta Åkesson
Karolinska Institutet
Institute of Environmental Medicine
Cardiovascular and Nutritional Epidemiology

Co-supervisors:
Risk-benefit assessor Jonas Toljander
Swedish Food Agency
Risk Benefit Assessment Department

Risk-benefit assessor Salomon Sand
Swedish Food Agency
Risk Benefit Assessment Department

Opponent:
Professor Manolis Kogevinas
Barcelona Institute for Global Health

Examination Board:
Senior lecturer Michael Fored
Karolinska Institutet
Department of Medicine
Division of Clinical Epidemiology

Professor Karin Källén
Lund University
Department of Clinical Sciences
Division of Obstetrics and Gynaecology

Associate professor Catherine Paul
Lund University
Department of Building and Environmental Technology
Division of Water Resources Engineering

To my family,

Klas, Björn and My

ABSTRACT

Drinking water is our most important food item and its access is indispensable for a well-functioning society. Recent Swedish water-borne outbreaks have demonstrated a system vulnerability and a clear need for improved knowledge on how variations in drinking water quality affects our health. This thesis explores this topic and consists of two large-scale epidemiological studies. The specific objectives were to i) obtain data on drinking water consumption patterns among adults, ii) assess whether changes in drinking water treatment and/or raw water source–aiming to increase pathogen reduction–affected the risk of gastrointestinal illness (GII) and iii–iv) assess if gestational exposure to by-products from drinking water chlorination war associated with the risk of adverse reproductive outcomes.

In a longitudinal cohort, we collected repeated information on tap water consumption and GII episodes via a monthly SMS among 5,200 participants during several periods in 2012-2016. The study was conducted in two parts of Sweden, in populations of neighbouring municipalities. In **Paper I**, we found that 99.8% of adults were consumers of cold tap water, while the consumption of bottled water was low. This lends support to the use of large register-based studies to assess the associations between drinking water exposures and health. In **Paper II**, we assessed if changes in municipal drinking water production and/or of raw water source affected the risk of GII. These changes encompassed either switching ground water treatment plant, changing from a surface to a ground water treatment plant or switching the surface water treatment plant and raw water source, all resulting in increased pathogen reduction in the drinking water. We observed no differences in the risk of GII among adults, however, among children, a 24% relative risk reduction in GII was observed after switching surface water treatment plant and raw water source. The indications that children are the most sensitive population to drinking water related GII are in line with previous findings.

In a nationwide register-based study, we assessed whether gestational exposure to chlorination by-products, trihalomethanes (TTHM), was associated with small for gestational age (SGA), preterm delivery or congenital malformations. We included more than 620,000 children born during 2005–2015 of mothers residing in Swedish localities (≥10 000 inhabitants) and where information on trimester specific TTHM exposure was available. The exposure was categorized into no chlorination, <5, 5–15, and >15 µg TTHM/L and stratified by treatment (hypochlorite and chloramine). In **Paper III**, we found indications of a dose-dependent multivariable-adjusted association of TTHM with risk of SGA in areas using hypochlorite, odds ratio (OR) 1.20 (95% confidence interval [CI]: 1.08-1.33) when comparing the highest exposed population to the unexposed. In **Paper IV**, TTHM was dose-dependently associated with malformations, but only in areas using chloramine. Comparing the population with highest exposure to the unexposed, ORs of 1.82 (95% CI: 1.07–3.12), 2.06 (95% CI: 1.53–2.78), 1.77 (95% CI: 1.38–2.26) and 1.34 (95% CI: 1.10–1.64) were seen for malformations of the nervous system, urinary system, genitals and limbs, respectively. The findings indicate that chlorination by-products may be associated with several adverse reproductive outcomes. Congenital malformations linked to chlorination by-product from chloramine use has not previously been highlighted and needs further attention.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Dricksvatten är vårt viktigaste livsmedel och en viktig del av ett fungerande samhälle. I dricksvattnet kan det dessvärre förekomma hälsoskadliga föroreningar, såsom mikroorganismer och kemiska kontaminanter. Kunskapen är dock begränsad om vilka föroreningar som kan vara hälsoskadliga och vid vilka halter dessa utgör en risk. Vi vet att sjukdomsframkallande mikroorganismer i dricksvatten kan orsaka magsjukeutbrott, men flera studier talar för att dricksvatten även kan bidra till icke-utbrottsrelaterad magsjuka, så kallad endemisk magsjuka. Studier har även indikerat att kemiska kontaminanter kan förekomma i hälsoskadliga nivåer. Utöver de som härstammar från råvattnet, kan kontaminanter även bildas i samband med dricksvattenberedningen, till exempel nedbrytningsprodukter från dricksvattenklorering. Det finns indikationer på att dessa nedbrytningsprodukter kan påverka hälsan, däribland ha en negativ effekt på fosterutvecklingen. Kunskapen kring dessa hälsoeffekter är dock ännu begränsad och resultaten ibland motstridiga.

I denna avhandling presenteras resultat från två olika epidemiologiska undersökningar. Den ena är en så kallade longitudinell kohort-studie, där deltagare under flera år regelbundet svarat på SMS-frågor. Den andra presenterar data från en landsomfattande registerbaserad studie, där medicinska och administrativa svenska register, samt dricksvattendatabaser, har använts som underlag. Avhandlingen bygger på fyra olika vetenskapliga arbeten som behandlar dricksvattenkonsumtionsmönster hos vuxna, sambandet mellan förändringar i dricksvattenberedningen och risken att drabbas av magsjuka hos vuxna och barn, samt sambandet mellan nedbrytningsprodukter från dricksvattenklorering och negativa effekter på fosterutvecklingen.

I en flerårig studie med ca 5 200 deltagare, samlades information om dricksvatten-konsumtion och magsjuka regelbundet in genom månatliga utskick av SMS-frågor. Baserat på resultaten från delar av studien, kunde vi se att nästan alla (99,8 %) vuxna deltagare drack kallt kranvatten och att de flesta (84 %) inte drack flaskvatten. Medelkonsumtion av kallt kranvatten var 1 L/dygn. Denna information kan användas som ett stöd i undersökningar där det saknas kunskap om studiedeltagarnas dricksvattenkonsumtion, exempelvis registerbaserade studier eller hälsoriskbedömningar. I studien undersökte vi även om tre olika förändringar i den kommunala dricksvattenberedningen och/eller byte av vattenkälla, påverkade risken att drabbas av magsjuka. Resultaten visade att risken för magsjuka hos vuxna inte påverkades av förändringarna, men att den relativa risken (kvoten mellan risken i studiedeltagarna från kommunen med förändringen och risken hos studiedeltagarna från grannkommunen som inte omfattades av förändring) för magsjuka hos barn minskade med 24 % i en kommun som bytte till grannkommunens dricksvatten (med en annan dricksvattenberedning och vattenkälla innebärande förbättrad reduktion av potentiellt sjukdomsalstrande mikroorganismer). Resultaten får stöd från flera tidigare studier, som indikerar att barn verkar vara en av de mest känsliga åldersgrupperna för att insjukna i dricksvattenrelaterad endemisk magsjuka.

I en studie som baserades på data från flera svenska register och databaser, utreddes om exponeringen för de fyra vanligaste nedbrytningsprodukterna från dricksvattenkloreringen,

trihalometaner (TTHM), påverkar risken att födas ”liten-för-tiden” (låg födelsevikt i relation till graviditetsveckan), för tidigt eller med missbildningar. I studien ingick alla nyfödda i Sverige under 2005–2015, vars mödrar hade bott i tätorter med mer än 10 000 invånare under sin graviditet och där det fanns tillräckligt underlag för att uppskatta mödrarnas TTHM-exponering under en för varje utfall relevant tre-månaders period av graviditeten. I studien ingick även mödrar som bott i tätorter där ingen dricksvattenklorering använts. Totalt inkluderades cirka 620 000 nyfödda. Resultaten indikerar att TTHM ökade risken för att barn föds ”liten-för-tiden” i de områden som använder kloreringsmetoden hypoklorit i dricksvattenberedningen, och att risken ökade med högre TTHM-halter. Inget sådant samband noterades hos barn till kvinnor som bodde i områden där kloreringsmetoden kloramin användes i beredningen. I de områden som använde kloramin, fanns i stället indikationer på ett samband mellan högre TTHM-halter och medfödda missbildningar på nervsystemet, urin- och könsorgan, samt skelett. Resultaten från studierna indikerar att nedbrytningsprodukter från dricksvattenkloreringen kan vara kopplade till flera negativa hälsoutfall under fosterutvecklingen. Eftersom resultaten var beroende av kloreringsmetod, finns det skäl att anta att det inte är TTHM, utan troligtvis andra, icke uppmätta nedbrytningsprodukter från kloreringen, som beskriver sambandet med dessa olika hälsoutfall. Negativa hälsoeffekter på fosterutvecklingen av nedbrytningsprodukter från kloramin har inte tidigare varit kända och behöver därför studeras ytterligare.

Sammanfattningsvis pekar resultaten från denna avhandling på att dricksvatten i Sverige kan bidra till endemisk magsjuka hos barn och att vi inte kan utesluta att exponering för nedbrytningsprodukter från dricksvattenkloreringen kan leda till negativa hälsoeffekter under fosterutvecklingen, även vid de låga halter vi har i Sverige. Denna kunskap är viktig för att kunna bygga upp arbetet med en förbättrad hälsoriskbedömning av dricksvattenberedningen, för att kunna väga risk mot nytta.

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III. **Säve-Söderbergh M.**, Toljander J., Donat Vargas C., Berglund M. and Åkesson A., 2020. Exposure to Drinking Water Chlorination by-Products and Fetal Growth and Prematurity: A Nationwide Register-Based Prospective Study. Environmental health perspectives 128(5): 57006.

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CONTENTS

LIST OF ABBREVIATIONS

CBP	Chlorination by-products
CI	Confidence interval
GII	Gastrointestinal illness
IPW	Inverse probability weighting
IRR	Incidence risk ratio
OR	Odds ratio
PTD	Preterm delivery
SGA	Small for gestational age
THM	Trihalomethanes
TTHM	Total trihalomethanes: sum of chloroform, bromoform, bromodichloromethane and dibromochloromethane
VPTD	Very preterm delivery

1 INTRODUCTION

Access to safe drinking water is essential for all humans to maintain health, but clean drinking water is also a key to reduce poverty, increase food safety, maintain peace and implement human rights (1). Access to safe drinking water is also the limiting factor to regional development, and key to a fully functioning society. While successful efforts have been made to increase the access to clean drinking water worldwide, one in three people still lack access to safe drinking water (1). As a result, one of the United Nations Agenda 2030 Sustainable Developmental Goals is therefore dedicated entirely to clean water and sanitation.

Even in countries like Sweden, where most people have access to drinking water of sufficient quality and sanitation, there are challenges. Due to persisting low water levels in ground water aquifers, raw water scarcity is an emerging problem. Drinking water producers therefore have to make the best of what is offered, which may result in costly solutions, especially if the alternative available water source is highly contaminated. Today we have the knowledge on how to produce drinking water from almost any water source available, even from seawater and sewage water. Still, producing drinking water is complex and several chemical and microbiological hazards have to be considered simultaneously. In addition, the distribution system must be supplied with sufficient quantities of drinking water during all hours of the day, every day of the year. When something fails in the treatment, the consequences will have impact on the entire society, like the drinking water related outbreak of *Cryptosporidium* in Östersund in 2010 (2). Outbreaks set aside; little is known on drinking water related health effects. For most pathogens and chemical contaminants, we still do not know which levels of contaminations that can be considered safe for human consumption, an alarming fact, as drinking water is the most consumed food item and commonly used in the food production.

This thesis will shed some light on potential adverse health effects of some drinking water related hazards, focusing on the most sensitive populations: unborn and children. In the studies included in this thesis, we assess if changes in the drinking water treatment affects the risk of endemic gastrointestinal illness and if drinking water related chlorination by-products are associated with adverse reproductive outcomes. To illustrate an example on how epidemiological findings may be used for decision-making, the findings were used in a preliminary risk-benefit assessment of using hypochlorite as drinking water treatment.

2 BACKGROUND

2.1 DRINKING WATER PRODUCTION

Before drinking water reaches the consumer by the distribution system, raw water–taken from a ground or surface water source–is treated in a water treatment plant. The drinking water treatment can consist of many treatment steps, depending on the occurrence of pathogens (disease-causing microorganisms) or of natural and anthropogenic contaminants in the raw water.

2.1.1 Drinking water treatment of pathogens

Several species of opportunistic bacteria, viruses and protozoans (parasites) have been detected in drinking water (3, 4). There are two ways of reducing these pathogens in the drinking water production: either by inactivation or by removal. Chlorination, ozonation or ultraviolet light are used for inactivation, while pathogens can be removed from the water through processes like flocculation and filtration (4). All treatment processes have different capacity to inactivate or remove microorganisms, commonly described by $\log_{10}$-reduction, i.e. the reduction of pathogens by a factor of ten. In practice, a $\log_{10}$-reduction of two would be equivalent to 99% of the pathogens being removed or inactivated. Due to high variance in size, and biological properties of pathogens, the $\log_{10}$-reduction of each treatment method may vary considerably between pathogens (5, 6). For example, while chlorine may successfully inactivate bacteria and viruses, it is inefficient when it comes to the protozoa (e.g. *Cryptosporidium*) (5). Therefore, to sufficiently reduce drinking water related pathogens, multiple treatment methods are often required. Multiple microbiological treatment will lower the risk of failure in the entire production and will increase the total $\log_{10}$-reduction of pathogens. However, interactions between treatments may occur, resulting in either increased or reduced microbiological risks (4).

2.1.2 Drinking water treatment of contaminants

The drinking water may contain both natural and anthropogenic contaminants. The type of contaminant that ends up in the raw water is affected by several factors, including physic-chemical properties of the contaminant, hydrogeological conditions of the aquifer and anthropogenic activities in the catchment area (7). The raw water may contain inorganic (metals, nitrogen species and natural constituents) and organic contaminants, some of which may be of anthropogenic origin (pesticides, solvents, etc.) (7). Drinking water contaminants can be removed by several treatment methods, like coagulation, activated carbon, membranes and filters. As with the pathogens, the efficient treatment methods are highly dependent on the properties of the contaminants, thus several chemical treatment methods are sometimes required to reduce contaminant levels.

2.1.3 Formation of chlorination by-product (CBP) during drinking water treatment

As chemicals are commonly used in the treatment of drinking water, the water treatment itself may be a source of chemical contamination. Chlorination is the most commonly used drinking water disinfectant globally and is also used in the majority of the water treatment plants in Sweden (8). Hypochlorite (sodium (Na) hypochlorite) and chloramine (monochloramine) being the most frequently used drinking water disinfectant. Hypochlorite is used to inactivate pathogens, while chloramine is mainly used to reduce/inactivate microbial growth on the distribution system. Due to strong oxidative properties, chlorine and other disinfectants efficiently inactivate pathogens, however the oxidative properties have an obvious downside, as chlorination by-products (CBP) are easily generated (9, 10). The formation of CBPs are regulated by temperature, pH, concentrations and composition of other substances in the drinking water, like natural organic matter and bromide, but also concentration of the chlorination treatment and contact time during the production. With large seasonal variation in environmental factors, like temperature and levels of organic matter, the CBP formation often follows a seasonal pattern (11). The maximum permitted concentrations of CBPs in drinking water are regulated by law in many countries and as a result, the four most common CBPs, the trihalomethanes (TTHM: chloroform, bromoform, bromodichloromethane and dibromochloromethane), are regularly monitored in municipal drinking water.

2.2 DRINKING WATER RELATED GASTROINTESTINAL ILLNESS (GII) AMONG CHILDREN

2.2.1 Mechanisms behind GII

Pathogen-induced GII is one of the most common diseases globally, especially among children (12). Children are more susceptible to pathogen exposure compared to adults. This is a result of children's immature neurological, immunological and digestive systems, reduced stomach acid and pepsin secretion, as well as having proportionally less extracellular fluids and a highly permeable intestinal mucosa (13, 14). Compared to adults, children are also at greater risk of becoming ill by a given dose of pathogens, and are often exposed to more pathogens due to their lack of sanitary habits and frequent mouthing behaviour (15). There is also a high proportion of asymptomatic carriers among children, which will contribute to the spread of the disease (16). Unfortunately, children are also at higher risk of developing severe GII illness, due to dehydration, as a result of rapid loss of body fluids and electrolytes (13). Most cases of childhood GII in high-income countries are, however, mild and the risk of mortality is low. Still, a high morbidity results in substantial societal costs, due to prevalent use of health care and parental absence from work. In Sweden, the risk of GII among children has been reported to be about two times higher than for adults, 0.8 and 0.4 cases/person year, respectively (17, 18).

The pathogens can induce GII symptoms by several mechanisms. Generally enterotoxigenetic bacteria (like pathogenic *Escherichia coli*), viruses and parasites affect the

small intestine, while invasive bacteria (like Campylobacter) affect the large bowel (19). The enterotoxigenetic bacteria will cause increased secretion of fluids from mucosal cells, resulting in watery diarrhoea, while invasive bacteria will penetrate the intestinal mucosa, often with bloody diarrhoea due to mucosal ulceration and inflammation. Exposure to some GII-inducing viruses (like Caliciviruses) result in mucosal damage and inflammation, although most mechanisms of increased fluid excretion due to virus-induced GII is not yet fully understood (19). Rotaviruses infect the small-intestinal enterocytes, interfering with the glucose-stimulated sodium pump, resulting in watery diarrhoea (20). Rotavirus also stimulates enterochromaffin cells (a type of enteroendocrine and neuroendocrine cell) in the gut, releasing 5-hydroxytryptamine, which affects the vagal afferents and eventually the brain stem vomiting centre, resulting in nausea and vomiting (21). In similarity with rotavirus infection, diarrhoea due to parasitic infection is a result of interference with the glucose-stimulated sodium pump (20). The infectious dose varies between pathogen species, ranging from a few, up to $>10^5$ organisms (22), but the infectious dose is also depends on the population at risk.

Fortunately, there are ways in which the body can protect itself from pathogen infection. To begin with, the established intestinal microflora will suppress any new microorganisms entering the system, by the production of antimicrobial substances such as bacteriocins and short-chained acids (19, 20). The composition of the intestinal microflora differs throughout the different parts of the digestive system, with cross-sectional differences as well (19). The mucosal epithelia also comprise innate defence mechanisms, the most important of which is the local formation and export of secretory immunoglobulin A antibodies (23). Secretory immunoglobulin A, together with additional defence systems, acts as the front-line for protection of the mucosa of the digestive system. There are many ways in which the mucosa can be infected, including colonization of the surface (non-invasive bacteria), epithelial penetration and replication in the body (invasive bacteria or viruses) and because of reaction with bacteria-released toxins. Therefore, developing vaccines is a challenge. In addition, the natural infections and mucosal immunization has been shown to be more effective in inducing secretory immunoglobulin A response compared to parenteral vaccines and the protection obtained from mucosal vaccination ranges from reduction of symptoms to complete inhibition of reinfection (23). Apart from vaccination and naturally gained immunity during childhood, newborns will receive acquired protection from maternal antibodies transmitted through the breast milk (23). However, for some pathogens, like norovirus, this protection will not last for more than the first 3–4 months of breast-feeding (24).

2.2.2 Current knowledge on drinking water related GII among children

Many raw water sources are contaminated by faecal pollution to some extent and if water is not sufficiently treated, faecal pathogens will end up in the drinking water (25-27). Given the many reported waterborne outbreaks, drinking water as the source of epidemic GII is fairly well known (28-31). In contrast, the contribution of drinking water to endemic GII cases is largely unknown (32, 33). It has been indicated that drinking water-related endemic GII may contribute to up to 35 % of all GII cases in developed countries, even in situations were

drinking water meets current quality criteria (32), however, there are also studies in which no indications of drinking water related GII have been observed (33).

Based on the results from epidemiological studies, there is increasing evidence that children are at highest risk of drinking water related endemic GII. Three observational studies have assessed the risk of GII by comparing populations in different areas with different drinking water treatments (34-36) while four studies, mimicking community interventions, assessed GII in relation to changes in the drinking water treatment for the same population (25, 37-39). Out of these, two studies showed a reduction in the number of cases of cryptosporidiosis when introducing membrane filtration (37) or coagulation and rapid gravity filtration (25) to the drinking water treatment. One study also showed a reduction in the incidence of GII, especially among children <5 years, when UV-disinfection was implemented in the water treatment process (38). In contrast, one study found no difference in the incidence for GII after the implementation of ozone and filtration into the water treatment process, although they demonstrated a significant reduction in the number of participants with ≥3 GII episodes after the change in water treatment (39). Studies have also assessed the effect of in-home filtration devices on GII incidence and although some of the studies have shown a reduction in the risk of GII (32, 40, 41), there are also studies indicating no effect (42, 43).

Assessing drinking water related endemic GII is challenging, especially due to the high impact of other sources of pathogen exposure. To begin with, most common pathway for GII related pathogens is person-to-person transmission through the faecal-oral pathway. This is especially profound for children due to their close social interaction, poor sanitary habits and the high risk of transmission because of a high proportion of children being asymptomatic carriers. Another challenge is linked to the case definition of GII. In epidemiological studies assessing drinking water related GII the case definition may vary from self-defined GII to a strict case definition, like laboratory-confirmed cases (25, 37, 38). The different case definitions have strengths and limitations. Using self-defined GII may introduce a recall bias and as not all GII symptoms are pathogen-induced, this method will compromise the sensitivity. While laboratory cases from medical records will reduce the risk of recall bias, only a fraction of all GII cases will seek medical care, thus potentially introducing another bias if hospital records are used as data source (44).

While the raw water is the most important source of drinking water related pathogens, it should be mentioned that pathogens might also be introduced during the distribution of drinking water. Studies have shown that breaks and pressure drops on the drinking water distribution system may increase the risk of GII with up to 60% and especially children seem to be affected (45, 46). Any event on the distribution system should therefore be considered a potential risk when assessing drinking water related GII.

2.3 CHLORINATION BY-PRODUCTS AND ADVERSE REPRODUCTIVE OUTCOMES

2.3.1 Prenatal development and adverse reproductive outcomes

The prenatal development, as well as the first years after birth, is generally the most sensitive period for exposure to contaminants during the lifetime (47). Most of the organ development occurs within distinct periods during the embryonic and the early foetal development. Not all organs develop simultaneously and the development of each organ has its unique set of biological mechanisms, often involving a complex signalling between neighbouring tissues (47). This results in organ-specific critical windows (Figure 1), associated with increased vulnerability to exposure to teratogens, e.g. chemicals, pathogens or other stressors (maternal trauma, metabolic imbalances, etc.), which in turn may affect the foetal development (48, 49). For some organs, the critical window begins even before the pregnancy can be confirmed, however, teratogens cannot cause adverse health effects before the start of the cellular differentiation (except for death of the embryo) (47, 50). Most major birth defects are introduced by exposure to teratogens during the organogenetic period (week 4–8), while physiological defects (e.g. minor morphological defects) are introduced during the foetal period (starting at week 9) (47). Exposure to teratogens may lead to changes in size, form or functionality of the organ, as well as changes in biochemistry and physiology, resulting in abnormal development (51). Besides the period of exposure to teratogens, other factors are relevant as well, including the dose and the genotype of the embryo and the mother (47). While the effects from exposure to teratogens are considered to have a dose-response relationship (increased exposure levels results in a more severe phenotypic effect), the genotype can affect the biological response to some teratogens and, as a result, only a fraction of the exposed embryos will develop a malformation (47). This may complicate the interpretation of epidemiological studies, especially as both the genotype of the mother or child and the mechanisms between genotype on the exposure-outcome interaction is often unknown. The most severe congenital malformations are likely to be diagnosed during the first years of life, while a large part of the less severe malformations will be discovered later in life or even go undiscovered (48, 51). In Sweden, the yearly prevalence of detected congenital birth defects and chromosomal abnormalities is around 3% of all newborns (about 3 cases/100 newborns), of which congenital heart defects is the most common (52).

During the second trimester, major cellular adaptions occur, followed by the third trimester when the organs mature, resulting in a rapid increase in body weight (47, 53). Exposure to maternal (e.g. genetics, malnutrition, diabetes), foetal (e.g. multiple births, impaired placental blood flow) or environmental factors (e.g. smoking, alcohol, infections) during the last period of the pregnancy, may therefore result in intrauterine growth restrictions (47, 53). The most common case definition for intrauterine growth restrictions, or impaired foetal growth, is small for gestational age (SGA). SGA arrives from the statistical distribution of birth weights by gestational age and sex, and refers to the lowest threshold of the curve for each week of gestation. In Sweden, <-2 standard deviation of the mean (2.3rd percentile) is generally used as the threshold for SGA, although SGA can be defined with other thresholds as well, 10th percentile being the most common (54). SGA has been

associated with increased perinatal mortality and morbidity (55). The majority of the children born SGA will accelerate in growth already during early childhood (56), however these children are still at risk of long-term effects of cardiovascular and metabolic diseases and chronic hypertension (57-60), as well as intellectual and educational outcomes (61).

Being born preterm is one of the most frequent cause of neonatal and childhood mortality and is a considerable contributor to the global burden of disease (62, 63) due to long-term morbidity, like neurodevelopmental impairment, respiratory problems and metabolic syndromes (64). A general case definition for preterm delivery is being born <37 weeks of gestation (PTD), but being born <28 weeks of gestation is also used as cut-off, often then referred to as very preterm delivery (VPTD). The majority of the PTD newborns are due to spontaneous preterm labour, but other causes are linked to complications due to multiple births, rupture of membranes, maternal hypertension, impaired foetal growth, antepartum haemorrhage, cervical incompetence or malformations of the maternal uterus (65). Several risk factors for PTD have been identified, including socioeconomic status, ethnicity, age, parity, drug use and infections (65).

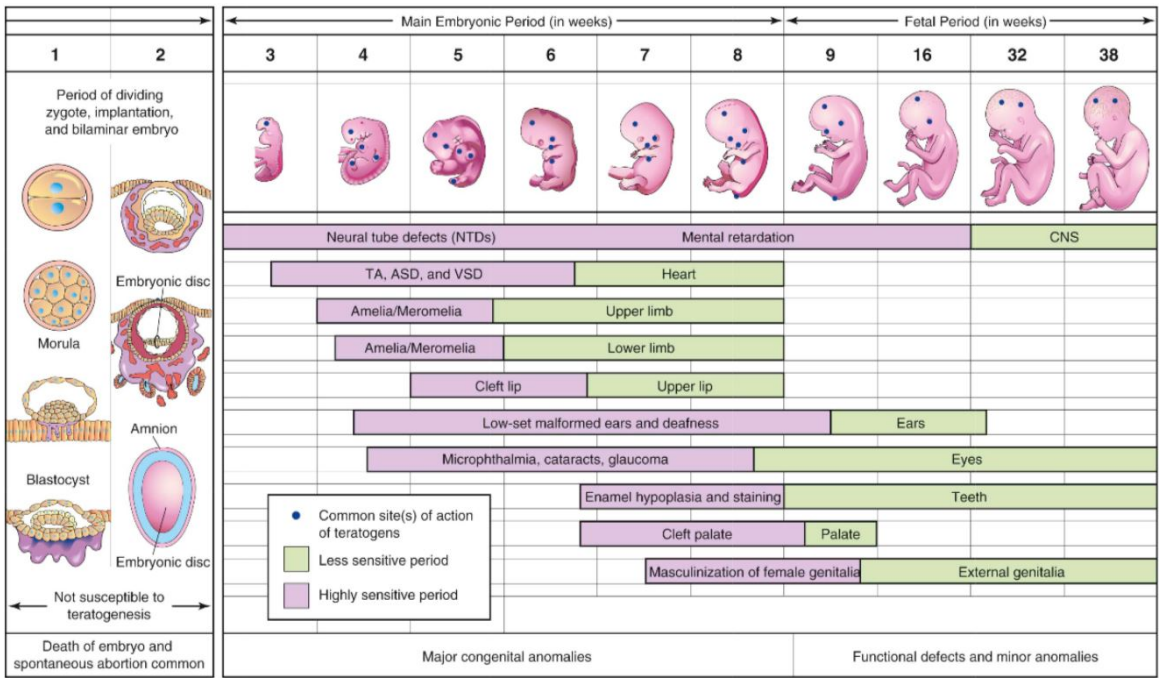

Figure 1 Critical periods in human development (published with permission from Moore KL, Persaud TVN. The Developing Human: Clinically Oriented Embryology (10th ed). Philadelphia: Saunders, 2016).

2.3.2 Current knowledge on chlorination by-product exposure and adverse reproductive outcomes

While there are some indications of an association for CBP exposure and the risk of adverse reproductive outcomes, the results are still inconclusive. The most consistent evidence is for intrauterine growth restrictions, where several studies have reported indications of an

association (66-74), however, an equal number of studies reported null findings (75-83). In addition, there has also been some indications of an association for CBP exposure and congenital malformations. While most epidemiological studies report null findings or even inverse associations (84-91), there are still some indications that neural tube (85, 92), urogenital (91, 93), limb (94) and heart defects (95-99) may be associated with CBP. Beside intrauterine growth restrictions and congenital malformations, there have also been indications that CBP exposure may be associated with spontaneous abortions (100), stillbirths (101-103) and preterm delivery (79, 84, 104, 105), however, several of the studies assessing these outcomes still report null findings (66-68, 75, 78, 80, 82, 106-111).

Several factors complicate the assessment of adverse reproductive outcomes associated with CBP in the drinking water. To begin with, there is a high risk of misclassification of the outcome. As most adverse reproductive outcomes are rare (112), often occurring among less than one percent of all newborns, limited statistical power is an imminent issue. An additional problem is the lack of homogeneity of the outcome (112). This has been raised especially for congenital malformations, where the aetiology behind the malformation may wary considerably, even for the same organ (47). The malformations included in the case definition may therefore highly affect the likelihood to detect an effect.

The most important limiting factors are however linked to the assessment of the exposure. Among the early epidemiological studies assessing CBP exposure and adverse reproductive outcomes, a crude exposure estimate was commonly used and the levels of CBP assessed were often unknown or limited. However, even among the more recent studies, where detailed data on tap water or internal CBP concentrations were used, challenges with the exposure persist. The most used indicator of CBP exposure has been the TTHM, either assessed as the sum or as individual THMs. THMs are highly volatile, thus resulting in a rapid absorption in the body, making showering, swimming, bathing and dish washing as relevant exposure pathways as the oral exposure (113, 114). Information on water related activities and behavioural patterns have therefore been collected and incorporated into the exposure estimate in some studies (82, 92, 100, 113-117) and some studies have even used urine and blood concentrations of CBPs to define the exposure (111, 118). Fortunately THMs have a short half-life in the human body, thus the internal dose is highly influenced by recent exposure, resulting in peak concentrations shortly after high TTHM exposure, like showering (119). In addition, CBP consists of mixtures and not all CBPs are volatile, complicating the exposure assessment even further (120). While tap water monitoring data has been most commonly used in the exposure assessments, the municipal tap water sampling strategies, as well as the spatial and seasonal variation in CBP levels (121, 122), are likely resulting in misclassification of the exposure. Drinking water monitoring data can be used for exposure assessment in small-scale distribution systems (123), however, the risk of exposure misclassification should still be considered (124). Even if exposure misclassification is reduced, regional variation in CBP formation will complicate the possibility to compare studies, as the highest exposure group in one study, may be the lowest exposure group in another. Therefore, null findings in one study may be the result of the CBP levels being below the levels of toxicological relevance, while a study with high CBP levels may not be

able to detect the full effect of the exposure, due to too high CBP concentrations in the reference group (too small exposure variation).

2.4 RISK-BENEFIT ASSESSMENT

2.4.1 Risks and benefits of drinking water chlorination

The three elements of risk analysis consists of risk assessment, risk management and risk communication (125). Risk assessment is the science-based part, in which to assess individual food related risks. However, sometimes both beneficial and harmful effects may be relevant to consider in parallel and this could be made through a risk-benefit assessment (126, 127). This is constructed in a similar way as the classical risk assessment with four steps, i.e. identification of adverse and beneficial effect, characterization of adverse and beneficial effect, exposure assessment and risk and benefit characterization including of the overall trade-off (126, 127). The risk-benefit assessment approach can be either qualitative, semi-quantitative or quantitative. In the quantitative approach, a common health metric is used, like disability-adjusted life years (DALY).

The first attempt to estimate risks and benefits of drinking water chlorination was made by Morris in 1978, in a semi-quantitative risk-benefit assessment (128) and was later raised in a qualitative risk-benefit assessment by Cotruvo in 1982 (129). However, since then, only one quantitative risk-benefit assessments has been made for drinking water chlorination, where reduction of waterborne infections was compared to the risk of cancer and adverse reproductive outcomes (130).

3 THESIS AIM

The overall aim of this thesis is to assess gastrointestinal illness and adverse reproductive outcomes in relation to exposures via drinking water.

The specific objectives:

Paper I–in a population-based longitudinal study, obtain data on drinking water consumption patterns among adults in Sweden, as support for health risk assessments in general and in particular for the exposure assessment in **Paper III–IV**.

Paper II–in a population-based longitudinal cohort, established to mimic a community intervention, assess whether municipal-level changes in raw water source and/or drinking water treatment affected the self-reported incidence of gastrointestinal illness among adults and children.

Paper III–in a nation-wide register-based cohort, assess the associations of gestational exposure to chlorination-by products in drinking water with the risk of being born small for gestational age, preterm or very preterm.

Paper IV–in a nation-wide register-based cohort, assess the associations of first trimester exposure to chlorination-by products in drinking water with the risk of congenital malformations.

4 METHODS

4.1 LONGITUDINAL COHORTS (PAPER I–II)

4.1.1 Study area and source population

The data in **Paper I** and **Paper II** originate from a longitudinal cohort in Sweden, consisting of several study parts, with the primary aim to assess the association between drinking water and the risk of endemic gastrointestinal illness. The data presented in **Paper I** was collected in the municipality of Ale in South-Western Sweden (Figure 2). Ale is an average sized municipality, with about 28,000 inhabitants and with a population equally distributed between the urban and the rural areas. Municipal drinking water was distributed from two water treatment plants, while the rural population received water from private wells. In **Paper II**, data was collected in two different areas: *i*) Falun and Borlänge in central Sweden and *ii*) Partille, and parts of Gothenburg in Southwest Sweden (Figure 3). These areas were selected based on planned changes of the raw water or water treatment. Falun and Borlänge are similar in size (about 40,000 inhabitants each) and at the baseline of the study; drinking water was distributed to each locality from two separate water treatment plants. Partille (about 80,000 inhabitants) received drinking water from their own water plant, whereas Gothenburg received drinking water from another water treatment plant, distributing water to 250,000 inhabitants in Gothenburg and other parts of the region (including parts of Ale).

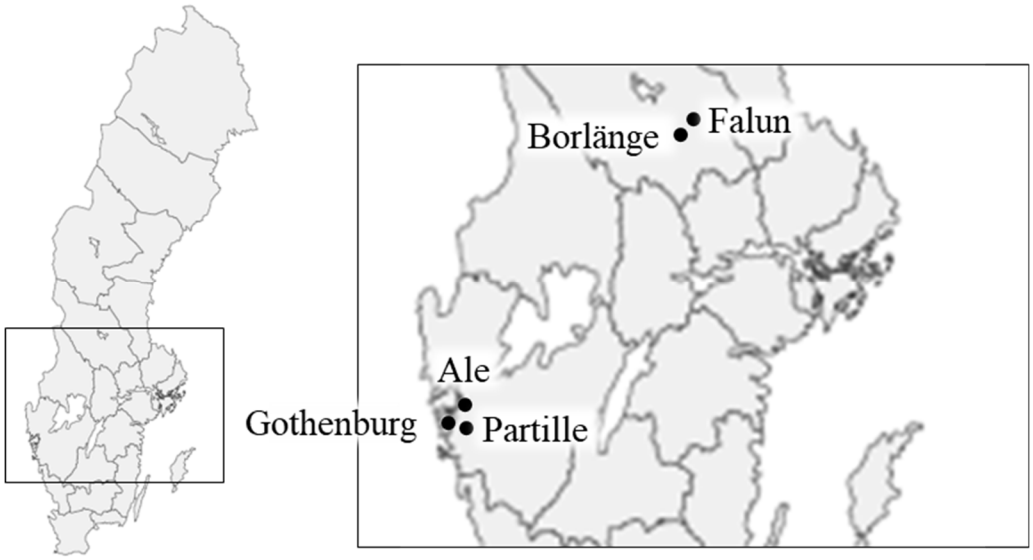

Figure 2 Geographical distribution of the areas included in the cohort in **Paper I-II** (map modified from Statistics Sweden).

4.1.2 Data collection

4.1.2.1 Telephone interviews

Participants were recruited to the cohorts by computer assisted telephone interviews (Figure 3). The consumer register was used to obtain a representative selection of adults from the study population (18–80 years), stratified by age and sex. During the interviews, background information, GII cases and symptoms, and tap water consumption was collected. Additional telephone interviews were also carried out during the study, to update personal information of the participants and to collect additional information, like tap water consumption among children (collected in November 2015 in Partille and Gothenburg). Informed oral consent was obtained from all participants.

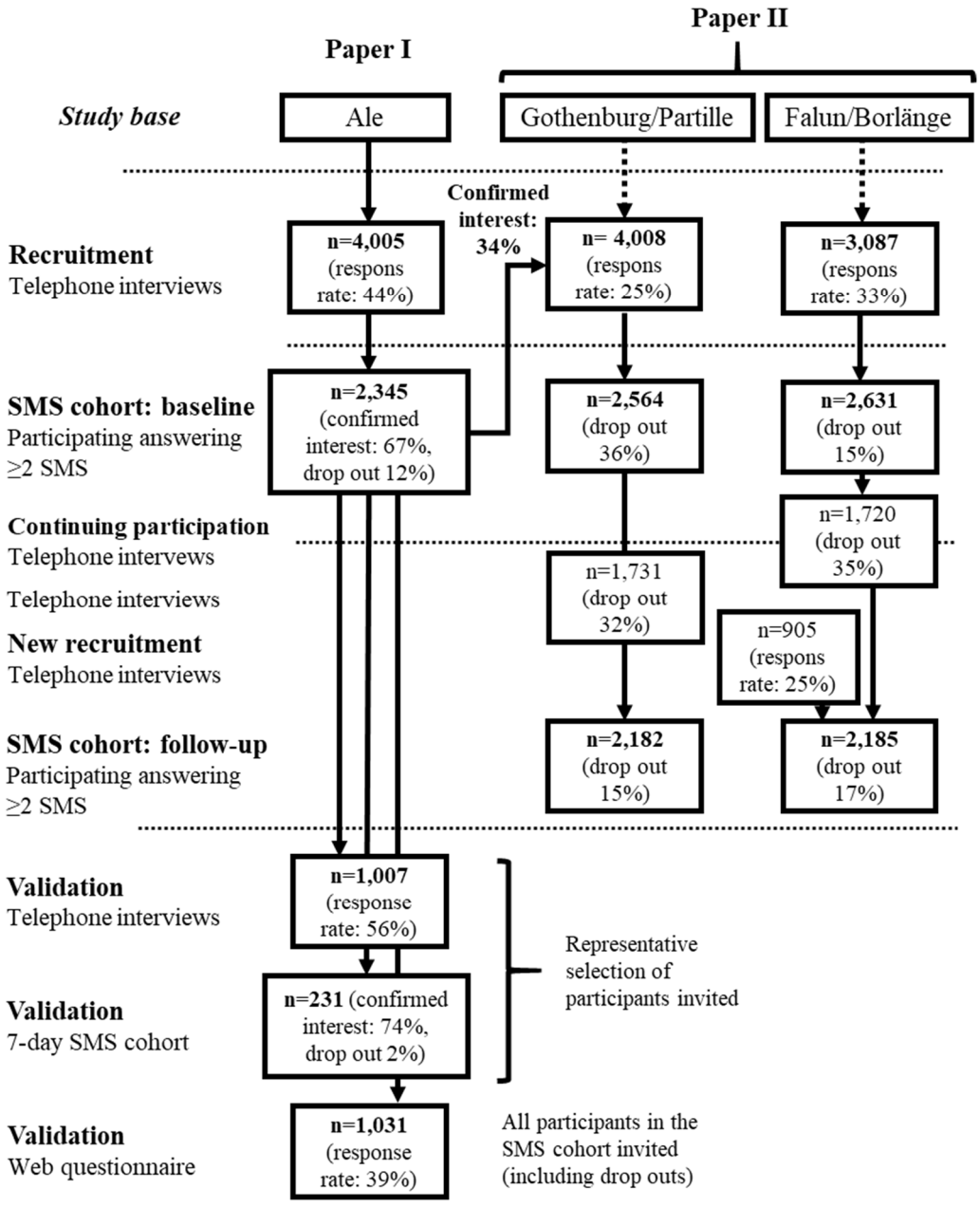

Figure 3 Overview of recruitment, SMS cohort and data validation in **Paper I–II**.

4.1.2.2 SMS questionnaires

Repeated monthly SMS questionnaires on tap water intake and GII were collected *i*) for descriptive statistics of the daily tap water consumption among adults in **Paper I** and *ii*) to assess if changes in water treatment and/or raw water affected the risk of GII among adult or children in **Paper II**. The data collection, time period and response rates are presented in Figure 3 andFigure 4. The recruited participants were categorized into two panels, equal in terms of age, sex, drinking water source and having children in the household. The panels received monthly SMS questionnaires during several study periods (each period being 9–13 months), either by the turn or the middle of the month, and the send-outs were evenly distributed between the days of the week over the entire data collection period. The total number of SMS sent out during the different data collection periods are presented inFigure 4. All participants were asked to report:

- the number of glasses of cold tap water they consumed during the last 24 hours (not including tap water used for cooking), and
- the episodes of GII during the last 28 days.

Participants having children aged 0–9 years in the cohort in Partille and Gothenburg were additionally asked to report:

- the number of vomiting/diarrhoea episodes among the household children aged 0–9 years during the last 28 days.

In case of illness during the last 28 days among the adults, participants received additional questionnaires on

- GII episodes during the last 14 days,
- description of symptoms during the last GII episode,
- number of loose stools and
- number of days of illness.

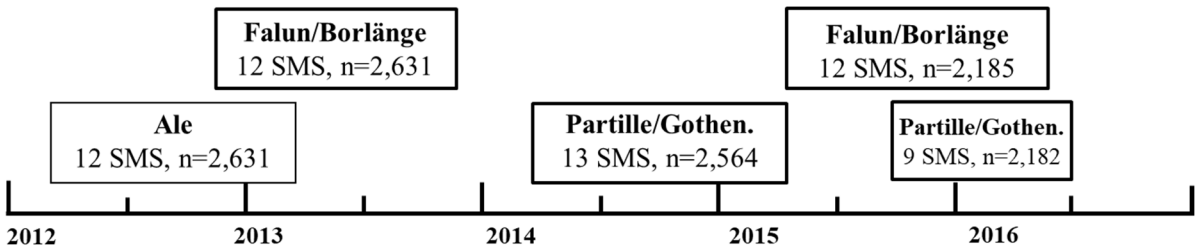

Figure 4 Overview of data collection periods of SMS in **Paper I–II** (SMS=number of monthly SMS send outs, n=numbers of participants answering at least one questionnaire).

All participants received a pre-reminder 24 hours before the questionnaires were sent out, reminding the participants to pay attention to their tap water consumption, and reminders were sent to those that had not completed all questionnaires. The SMS questionnaires were sent at 10 a.m. and the participants had 48 hours to answer all SMS questionnaires. When the monthly SMS questionnaires were completed, participants were reimbursed with a lottery scratch card, each (**Paper I**) or every second month (**Paper II**). In **Paper I**, all participants were included throughout the study period, only excluding self-initiated exclusion. In **Paper II**, participants were excluded in case of non-response for three succeeding months. Participants were also reminded to report if they had moved. In case they had moved within the study area, the participants were reclassified, if needed, and if they had moved outside of the study area, they were excluded.

4.1.3 Changes in raw water and water treatment (Paper II)

In **Paper II**, the study design was mimicking a community intervention, in which three drinking water related changes were assessed (Figure 5):

- Change 1, when a new ground water treatment plant with UV disinfection was introduced in Borlänge,
- Change 2, when Falun changed from using its own surface water source, to receiving drinking water from the new ground water treatment plant in Borlänge, and
- Change 3a (adults) and 3b (children), when Partille changed from using the municipality's own surface water treatment plant to receiving water from the water treatment plant in Gothenburg.

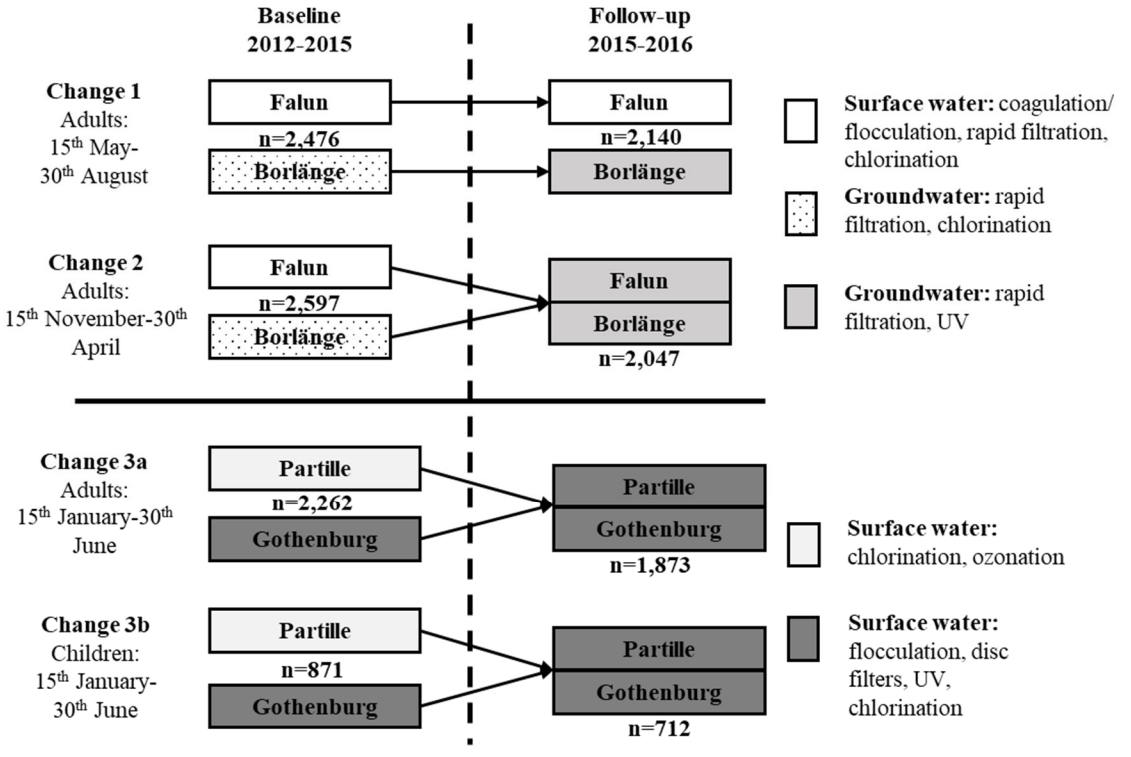

Figure 5 Schematic overview of drinking water related changes in **Paper II**.

Change 1–2 only included adult participants, while Change 3 included both adults (3a) and children (3b). No additional biological samplings were initiated during the study period, neither among the participants, nor of the drinking water, however, municipal monitoring data of indicator organisms was available for the raw and tap water. This also meant that we did no additional measurement of community-level changes in pathogen reduction by drinking water treatment.

Effort was put into the study design to reduce the impact of non-drinking water related GII. To reduce influence from any travel related GII, participants were asked not to report GII episodes in case of travel for more than a week during the 28-day recall period. Other information collected and considered when assessing the study results were differences in the seasonal trend of winter vomiting disease in Sweden during the study period, possible effect by a newly introduced rotavirus vaccination programme in Partille, any differences in the rate of pipe breaks and other drinking water related events, like treatment failure, as recorded by the drinking water utilities.

4.1.4 Outcomes

In **Paper II**, participants were asked to report self-defined GII for themselves and pre-defined case definition of GII for their children. Participants were informed that seven disease-free days must have passed between two separate GII episodes. In **Paper II** the following case definitions were used:

- self-defined GII among adults (recall 28 days, any symptoms),
- acute GII (AGI: recall 28 days, vomiting and or three loose stools during 24 hours), and
- vomiting and/or diarrhoea occurring among household children aged 0–9 years (recall 28 days).

If a participant replied only to the initial question of water consumption, it was assumed that the respondent had not suffered from GII during the last 28 days in the statistical analyses.

4.1.5 Covariates

In the cohorts included in **Paper II**, covariates were collected or updated during telephone interviews. The data collected in Ale was used to estimate drinking water related GII and to validate SMS as a tool for GII and tap water consumption (**Paper I**), but also to estimate relevant risk factors for the succeeding parts in the cohort (**Paper II)**. Based on the results from Ale, the following covariates were considered relevant for adults in **Paper II**:

- sex,
- age, and
- children 0–5 years in the household (yes/no).

As children were included in the cohort in Partille and Gothenburg, additional background information was collected:

- educational level,
- employment status,
- voting in democratic elections,
- economy,
- occupation with increased risk of pathogen exposure (day-care, school, youth recreation center, medical care, retirement home or sewage-related work),
- number of children in the household and number of children at day-care.

4.1.6 Statistical analyses

Paper I consists of descriptive statistics of the daily tap water consumption among adults. We use Kruskal-Wallis test to assess difference in the tap water consumption between groups based on age and gender, Spearman's rank test to estimate correlation between single consumption estimates and the average consumption and random-effect generalized least square regression to estimate the intra-individual correlation from the responses in the cohort. Non-parametric test was used, as the data on tap water consumption was not normally distributed. Single tap water estimates exceeding 30 glasses/24 h (6 l/24 h) were excluded, as were participants with an average daily cold tap water consumption exceeding 20 glasses (4 l/24h) (**Paper I**–**II**).

In **Paper II**, we assessed the impact of drinking water related changes on population level GII. By estimating the ratio of the incidence rate ratios (IRR) of GII in the two neighbouring areas (Falun/Borlänge and Partille/Gothenburg) before (baseline) and after the change (follow-up) ($IRR_{follow\text{-}up}/IRR_{baseline}$). We used the same calendar time-specific periods for baseline and follow-up. We used Poisson regression for adult GII data and negative binomial regression (random effect) for GII among children. The selection of Poisson regression or negative binomial regression was based on distribution of GII data for adults and children. The Poisson model for adult data (Change 1–3a) was adjusted for the following covariates age (≥55/<55), gender (male/female), having children aged 0–5 years (yes/no) and the individually reported water consumption (quartiles). All reported events of self-defined GII or AGI were summed per participant and the number of SMS responses was treated as offset (total person-months). Negative binomial regression model for GII among children 0–9 year (Change 3b) was adjusted for children at day-care within the household (yes/no) and the model was pooled by household. Although average tap water consumption for each child was collected during telephone interviews, it was not considered in the regression model, as GII data was aggregated on household level. Individual SMS responses >10 GII episodes among children were excluded (0.03% of SMS questionnaires). To estimate the impact of non-response between baseline and follow-up (assuming missing at random), $IRR_{follow\text{-}up}/IRR_{baseline}$ was calculated using inverse probability weighting (IPW) (131), balanced for child at day-care in the household. The relative risk reduction of GII during the calendar time-specific comparison of interannual periods was calculated: $100 \cdot (IRR_{baseline}\text{-}IRR_{follow\text{-}up})/IRR_{baseline}$. Chi^2 test was used to assess differences in respondent characteristics. Statistical analysis in **Paper I** and **Paper II** was performed using R version 3.2.3 (R Core Team, 2015,

R Foundation for Statistical Computing, Vienna, Austria) or Stata 14.1 (StataCorp, Texas, USA) and statistical significance level was set at 0.05.

4.2 REGISTER-BASED COHORTS (PAPER III-IV)

4.2.1 Registers and databases

The data used in **Paper III** and **Paper IV** originates from Swedish health care and administrative registers: the Swedish Medical Birth Register, Longitudinal Integration Database for Health Insurance and Labour Market Studies (commonly known as LISA) and a national register for regional divisions based on real estate (Geografidatabasen). Exposure data, i.e. information of tap water content, was obtained from a nation-wide database on drinking water analyses (Vattentäktsarkivet) and information of water treatment was received from publications by the national association for drinking water producers (8, 132).

The Medical Birth Register was founded in 1973 and is administered by the National Board of Health and Welfare. The register includes records from the antenatal care of the mother, the delivery record (from gestational week 22) and the record of paediatric examination of the newborn infant (newborn period: up to 28 days after birth), as well as a selection of information from national administrative records. As maternal and delivery care are publicly funded in Sweden and as notification to the Medical Birth Register is mandatory for health care personnel, information on almost all newborns in Sweden is included in the register (133).

Longitudinal Integration Database for Health Insurance and Labour Market Studies is administered by Statistics Sweden and is a longitudinal register founded in 1990 containing personal information for all registered Swedish citizens age ≥16 years, including demographic, civil status, migratory information, education, family demographics, occupation, income, sick leave, etc. The individual variables in the registers have a coverage of 80–98% (134). Statistics Sweden also administers Geografidatabasen, a longitudinal register containing regional divisions of the property stock that could be linked to registered residence of Swedish inhabitants, in order to obtain information on residential history.

Vattentäktsarkivet is administrated by the Geological Survey of Sweden and contains technical information on water sources and drinking water production facilities, as well as results from municipal drinking and raw water monitoring. The information has been collected since 2002; however, most drinking water data was only available from 2005 to 2014. The database contains analytical results from about one million tap water samples.

4.2.1 Study area and source population

An overview of the identification of study area (Figure 6) and study population, as well as data linkage is presented in Figure 7. To have comparable study areas with regard to size, exclusively having municipal drinking water, we selected all localities (a regional division, defined by a continuous populated area with at least 200 inhabitants) in Sweden having a population exceeding 10,000 inhabitants (116 localities, in which about 60% of the Swedish

population have their residence). Based on available CBP monitoring data in Vattentäktsarkivet, the period 2005–2015 was selected for register linkage. Localities were excluded if:

- there had been changes in the drinking water chlorination treatment methods (8, 132) during 2005–2015,
- TTHM was detected in the drinking water although no chlorination was used in the production,
- the localities received drinking water from more than two treatment plants or two water treatment plants with a mean difference of <10 µg/l in CBP concentrations, or
- CBP had been analysed less than four years between 2005 and 2015.

In total, 83 localities were included as study area.

The study population was identified as children born 2005–2015, with mothers having their home address registered at one of the selected localities during any part of their pregnancy. This was done by linking information on births during 2005–2015 from the Medical Birth Register, to Geografidatabasen for identification of maternal home address at birth, 6 months prior to birth and 10 months prior to birth. The linkage was possible due to the mother's personal identification number, a unique number assigned to all Swedes and generally used for identification in registers (135). Additional linkage by the personal identification number was performed by Statistics Sweden to the Medical Birth Register and the Longitudinal Integration Database for Health Insurance and Labour Market Studies, to obtain health care and administrative data. Multiple births (**Paper III** only) and mothers reporting occupations with high CBP exposure (professional swimmer, coach to professional swimmers or employment at swimming pool facilities) were excluded (Figure 7).

Figure 6 Localities included as study areas in **Paper III**–**IV** (Map modified from statistics Sweden).

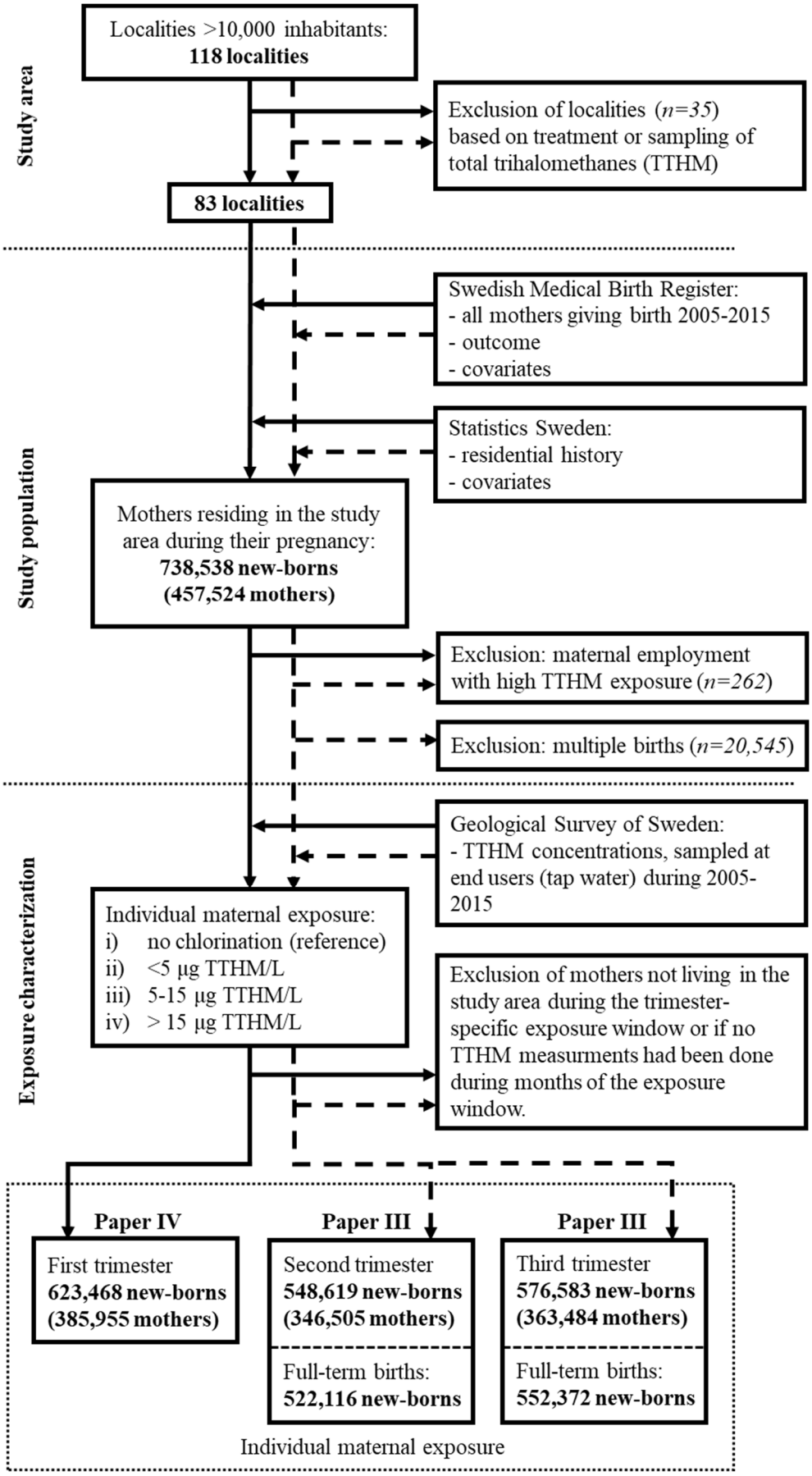

Figure 7 Study area, study population and exposure categorisation in **Paper III–IV**.

4.2.2 Exposure

We used the sum of water tap concentrations of TTHM sampled at end users in the distribution system. The assigned exposure category was based on a trimester-specific, three-month average for each mother that had been living in the locality during the trimester-specific three months (1st, 2nd or 3rd trimester). To account for the seasonal variation and inter-annual deviations in the municipal tap water monitoring program, we used a multiannual TTHM exposure. The exposure estimate was generated by a multiannual and locality-specific monthly average of TTHM based on samples as end users. The average TTHM was then used to estimate a trimester-specific (three months) average for each pregnancy. The relevant trimester of exposure for the statistical analyses was based on prior knowledge of the effect-window of the outcomes:

- 1st trimester: congenital malformations,
- 2nd trimester: SGA, PTD/VPTD,
- 3rd trimester: SGA

The individual maternal exposure was categorized into:

- no chlorination,
- <5 μg TTHM/L,
- 5–15 μg TTHM/L,
- >15 μg TTHM/L

4.2.3 Outcome

In **Paper III**, we used SGA, PTD (born before gestational week 37) and VPTD (born before gestational week 32) as outcomes. All outcomes were restricted to singleton births and for SGA only full term births were included. SGA was pre-defined by the health care as <-2 standard deviation from the average weight and gender for the gestational age at partus (136). The general recommendations in Sweden for estimating gestational age is by fetometry (i.e. measurement of the size of the foetus) during ultrasound at gestational week 11–22 (137, 138), thus the majority of the estimates for gestational age were derived from ultrasound evaluations, while the last menstrual period was used to define the gestational age for a minor part of the estimates.

In **Paper IV**, we included congenital malformations grouped by major malformation according to the International Classification of Diseases 10th Revision (ICD-10) codes (Table 1) and defined by major malformations according to the European Surveillance of Congenital Anomalies (139). Major malformations with a prevalence of <0.5/1000 births were not assessed, thus excluding malformations of the eye, face and neck, ear, respiratory system and abdominal wall.

Table 1 The International Classification of Diseases 10th Revision (ICD-10) codes for major congenital malformations as classified by European Surveillance of Congenital Anomalies.

Major congenital malformations	ICD10	Excluded ICD10
Nervous system	Q00-Q07	Q0461, Q0782
Eye	Q10-Q15	Q101-Q103, Q105, Q135
Ear, face and neck	Q16, Q17, Q18	Q170-Q175, Q179, Q180-Q182, Q184-Q187, Q1880, Q189
Heart Defects	Q20-Q26	Q2111, Q250 if PTD, Q2541, Q256 if PTD, Q261
Respiratory	Q300, Q32-Q34	Q314, Q315, Q320, Q331
Oro-facial clefts	Q35-Q37	
Digestive system	Q38-Q45, Q790	Q381, Q382, Q3850, Q400, Q401, Q4021, Q430, Q4320, Q4381, Q4382
Abdominal wall defects	Q792, Q793, Q795	
Urinary	Q60-Q64, Q794	Q610, Q627, Q633
Genital	Q50-Q52, Q54-Q56	Q523, Q525, Q527, Q5520, Q5521
Limb	Q65-Q74	Q653-Q656, Q662-Q669, Q670-Q678, Q680, Q6810, Q6821, Q683-Q685, Q7400
Chromosomal	Q90-Q92, Q93 , Q96-Q99	Q936

4.2.4 Covariates

We selected potential confounders based on prior knowledge on risk factors (Table 2, Table 3). The same risk factors were considered relevant for SGA and PTD/VPTD (140, 141). The same strategy was used for all major congenital malformations.

The following covariates were selected for SGA and PTD/VPTD:

- maternal age (<25, 25–<30, 30–<35, 35–<40, ≥40 years),
- BMI (at registration to antenatal care: <18.5, 18.5–<25, 25–<30, ≥30 kg/m^2),
- birth region (Nordic/Europe/Africa/North and South America/Asia/other),
- attained education (elementary school/secondary/post-secondary education),
- household income (yearly quartiles by year of birth),
- smoking at gestational week 30–32 (no, 1-9 cigarettes/day, >9 cigarettes/day),
- previous miscarriages (yes/no),
- parity (nulliparous, 1, 2, ≥3),
- sick leave/being on disability,
- use of teratogenic drugs (yes/no) (142),
- maternal illness: maternal diabetes (yes/no), preeclampsia (yes/no), maternal hypertension (yes/no), maternal weight gain (high/normal/low weight gain) and
- year of birth (continuous).

Table 2 A selection of risk factors identified for small for gestational age (SGA) and preterm delivery (PTD).

Risk factor	Direction of the association	Reference
Maternal demographics		
Increased age	↑	(65, 143-145)
Ethnicity	*	(65, 143, 146, 147)
Maternal medical conditions		
Maternal weight	↓	(143, 144, 148)
Diabetes	↑	(143, 149)
Preeclampsia	↑	(145, 150)
Hypertension	↑	(65, 143, 145)
Maternal socioeconomic disparities		
Income	↑	(65, 151, 152)
Education	↓	(65, 145, 151, 152)
Obstetric history		
Previous miscarriages	↑	(143, 153)
Nulliparous	↑	(143, 144)
Maternal substance exposure		
Smoking	↑	(143, 145, 148)
Teratogenic drugs	↑	(144, 154)

↑ increased risk, ↓ inverse association, * higher risk among African-American compared to Caucasian.

The following covariates were selected for congenital malformations:

- maternal age (<25, 25–<30, 30–<35, 35–<40, ≥40 years),
- BMI (at registration to antenatal care: <18.5, 18.5–<25, 25–<30, ≥30 kg/m^2),
- attained education (elementary school/secondary /post-secondary education),
- household income (yearly quartiles by year of birth),
- smoking at registration to antenatal care (no, 1–9 cigarettes/day, >9 cigarettes/day),
- parity (nulliparous, 1, 2, ≥3),
- use of teratogenic drugs (yes/no) and
- maternal diabetes (yes/no).

Table 3 A selection of risk factors identified for congenital malformations.

Risk factor	Malformations associated to risk factor	Reference
Maternal demographics		
Increased age	NS, CHD, DS, GD, LD, CA	(155-160)
Maternal medical conditions		
Maternal weight	NS, CHD, OFC, DS, GD, LD	(156, 160-162)
Diabetes	NS, CHD, OFC, UD, DS, GD, LD	(155, 156, 160, 163)
Socioeconomic disparities		
Income /education	NS, CHD, OFC, CA	(157, 164, 165)
Obstetric history		
Increased parity	NS, CHD	(155, 157, 159)
Maternal substance exposure		
Smoking	NS, CHD, OFC, DS, UD, LD	(155-157, 159, 160, 166)
Teratogenic drugs	NS, CHD, OFC, DS, UD, GD, LD, CA	(160, 167)

NS=Nervous system, CHD=Congenital Heart Defects, OFC=Oro-facial clefts, DS=Digestive system, UD=Urinary, GD=Genital, LD=Limb, CA=Chromosomal

4.2.5 Statistical analyses

In **Paper III–IV** we used logistic regression to estimate the odds ratio (OR) and 95% CI of the outcome for each exposure category. The analyses were clustered (intragroup correlation) for the same mother. We used standardization (IPW) to adjust for confounding. For SGA, PTD and VPTD, two multivariable-adjusted models were assessed, in which maternal age, BMI, birth region, highest attained education, household income and smoking were considered the most relevant and therefore included in the Model 1. Previous miscarriages, parity, sick leave/being on disability, use of teratogenic drugs, maternal illnesses and year of birth were additionally included in Model 2. For congenital malformations, only one multivariable-adjusted model was used, including all relevant identified confounders. Based on the median TTHM concentrations for each exposure category, we generated a continuous variable to estimate the linear trend. In additional analyses, we stratified the exposure by chlorination procedure (hypochlorite only or chloramine only, as reported in 2010). For hypochlorite, only hypochlorite used as primary disinfection was included (i.e. to inactivate microorganisms during the water treatment), while for chloramine, we included both prepared monochloramine, but also when a combination of hypochlorite and ammonia had been used to generate monochloramine as secondary disinfection (i.e. to reduce microbial growth on the distribution system). In a sensitivity analyses, we used the lowest exposure category (<5 µg TTHM/L) as reference, to account for potential unmeasured confounders linked to using/not using chlorination as a treatment procedure and because some previous studies used this group as the reference due to the lack of populations exposed to non-chlorinated municipal drinking water. All statistical analyses were performed using Stata 14.1 (StataCorp, Texas, USA) and statistical significance level was set at 0.05.

4.3 IMPLEMENTATION OF THE RESULTS FROM PAPER II-IV INTO A RISK-BENEFIT ASSESSMENT OF HYPOCHLORITE

The results from **Paper II–IV** were used in a risk-benefit assessment of hypochlorite as drinking water treatment (**Appendix 1**), according to the procedure described by the European Food Safety Authority (127). Focusing on Swedish conditions and both ends of the extreme conditions during normal operation, the following scenarios will be assessed:

- *Scenario 1:* High pathogen inactivation by hypochlorite and low TTHM formation
- *Scenario 2:* Low pathogen inactivation by hypochlorite and high TTHM formation

Pathogen inactivation by hypochlorite quantified as the lowest and highest weighed theoretical $\log_{10}$-reduction. The methods for generating the weights are presented by Tornevi et al. (2016) (36), and are based on the mean value of the $\log_{10}$-reductions for the water treatment for bacteria, virus and parasites, where each pathogen group is weighted based on how commonly these pathogen groups cause GII (5, 175). For hypochlorite the weighed theoretical $\log_{10}$-reduction was estimated to be 2.05 for the low pathogen inactivation and 4.26 for the high pathogen inactivation (estimated theoretical $\log_{10}$-redution for hypochlorite: virus 2.5–5.0, bacteria 1.5–3.0, protozoa 0–1.0; estimated pathogen weights: virus=0.79, bacteria=0.05, protozoa=0.16) (5, 168). TTHM formation is based on TTHM levels reported

in **Paper III–IV**, thus <5 µg TTHM/L for low TTHM formation and ≥15 µg TTHM/L for high TTHM formation.

To estimate the beneficial effect of hypochlorite, the results from **Paper II** were used to generate the dose-response for the $\log_{10}$-pathogen reduction and GII incidence. To estimate the dose-response, we considered i) change in pathogen reduction (using the theoretical $\log_{10}$-reduction (5, 168)) and ii) change in pathogen levels (**Paper II**). As the water treatment plants in Sweden have a high pathogen reduction, we can assume the pathogen exposure to be low, thus a linear extrapolation was used (169). Based on this we assess a slope (m) of the log-linear dose-response of the $\log_{10}$-reduction (**Paper II**) to calculate the yearly theoretical reduction of GII cases as a result of using hypochlorite.

To quantify the burden of disease from mortality and morbidity, DALY was used (*Equation 1*). DALY is estimated by the sum of Years Lived with Disability (YLL, *Equation 2*), which is linked to premature death and Years of Life Lost (YLD, *Equation 3*), which holds information on severity, incidence and duration.

$$DALY = YLL + YLD \qquad \textit{(Equation 1)}$$

$$YLL = N \cdot L \qquad (\textit{Equation 2})$$

where N is the number of deaths, L is the standard life expectancy at age of death in years, and

$$YLD = P \cdot D \cdot DW \qquad (\textit{Equation 3})$$

where P is the number of cases, D is the duration in years and DW is disability weight.

All data on prevalence or incidence of disease was obtained from **Paper III-IV**. National data on deaths for the relevant disease was obtained from The Swedish Cause of Death Register administered by National Board of Health and Welfare (170) and demographics for Statistics Sweden (171). A life expectancy of 89.1 years was used (Swedish women in 2060) (172). For comparison of estimates, we used the nationwide estimates of the population at risk of exposure for high TTHM levels (>15 µg TTHM/L). It was estimated that the population in Sweden potentially exposed to >15 µg TTHM/L every year were 100,000 children 0–9 years and 10,000 newborns.

For GII, a disability weight of 0.074 and a duration of 0.01 years (3.5 days) was used for each GII episode. For SGA, a disability weight of 0.8 was used, based on extreme low birth (500–999 g) weight with mild neurological disability and with a duration of one year (173, 174). No long-term effects of the outcomes were considered, as this was not assessed in **Paper III-IV**.

5 RESULTS

5.1 DRINKING WATER CONSUMPTION PATTERNS (PAPER I)

The main findings in **Paper I** indicated an average daily cold tap water consumption of 1 L/day (median: 0.92 L/day; interquartile range: 0.64–1.28 L/day) for adult Swedes. The participants with the highest consumption (99th percentile), reported an intake of 2.5 L/day. About 70% of the cold tap water was consumed at home and the average total consumption of heated and unheated tap water was 1.85 L/day (not including water used for cooking). About 99.8% of the respondents reported to be consumers of cold tap water and about 84% reported to be non-consumers of bottled water. The tap water consumption was higher among women compared to men. Among women of childbearing age (18–49 years), the mean consumption ranged between 0.84–1.14 L/day, with the lowest consumption in the youngest age groups and increasing with age.

The children in Partille and Gothenburg in **Paper II** (data not shown in the paper), reported a mean consumption of cold tap water of 0.3 L/day, 0.7 L/day and 0.8 L/day for children <2 years, 2–5 years and 6–9 years, respectively. Most children (97%) reported to be consumers of cold tap water.

5.2 GASTROINTESTINAL ILLNESS AND CHANGE OF RAW WATER AND DRINKING WATER TREATMENT (PAPER II)

During Change 1–3a in **Paper II**, about 2,500 adults were included during baseline, while about 2,100 were included during follow-up. During Change 3b, 871 and 708 children were included during baseline and follow-up, respectively. The few differences in the population characteristics between the neighbouring municipalities were generally consistent between baseline and follow-up.

The average yearly GII incidence among adults was 0.36 episodes of AGI/person-years, while there were 1.45 episodes of vomiting and/or diarrhoea/person-year among children. The drinking water related changes included in **Paper II** did not have a significant effect on GII or AGI among adults for neither of the assessed changes, however, for Change 3b, a 24% reduction in vomiting and/or diarrhoea was observed for children 0–9 years (Table 4).

Table 4 Multivariable-adjusted incidence rate ratio (IRR) of GII (vomiting/diarrhoea) among children during baseline and follow-up, as well as their ratio (IRR-ratio) for Change 3b (change of water treatment plant in Partille).

	Analyses	**IRR (95% CI)**
Change 3b	Baseline (n=871)	1.19 (0.99–1.42)
	Follow-up (n=712)	0.90 (0.68–1.17)
	IRR-ratio	0.76 (0.59–0.98)
	IRR-ratio (IPW)	0.71 (0.47–1.08)

Models adjusted for child at day care. IPW: inverse probability weighting.

5.3 CHLORINATION BY-PRODUCTS AND ADVERSE REPRODUCTIVE OUTCOMES (PAPER III–IV)

In total, 548,619 and 576,483 singleton births were ascertained for second and third trimester exposure, respectively (Figure 7). Among these, about 1.9% were diagnosed as SGA, 4.7% as PTD and 0.7% as VPTD (**Paper III**). When including multiple births, 623,468 newborns were ascertained for first trimester exposure. Among these, the total prevalence of congenital malformations was ~2/100 births, with heart defects being the most common (~8/1,000 births) (**Paper IV**).

For all populations assessed, we observed some differences across exposure groups in the population characteristics. Within the population included in **Paper III,** there were some differences between the exposure groups on maternal age, country of birth, attained educational level, household income and sick leave/disability pension. In **Paper IV,** there were mainly differences in maternal age, attained education and household income. Beside differences in the population characteristics, there were also some locality-specific differences. As expected, for both **Paper III** and **Paper IV**, the majority of the non-chlorinated areas used ground water as their raw water source. Ground water was also common in areas using hypochlorite, but uncommon in areas using chloramine. In addition, the areas receiving non-chlorinated drinking water were generally smaller, as compared to the chlorinated areas and as expected, this was especially profound for areas using chloramine.

5.3.1 Small for gestational age and preterm delivery (Paper III)

5.3.1.1 Small for gestational age

When not differentiating by chlorination treatments, no association was seen for SGA among newborns and third trimester TTHM exposure. When stratifying the analyses by chlorination treatment, SGA was significantly associated with TTHM in areas using hypochlorite, OR 1.20 (95% CI: 1.08-1.33, p-trend: <0.001, Table 5), comparing the population in the highest TTHM exposure (>15 µg TTHM/L) to the unexposed population. The association remained when the lowest TTHM exposure group (<5 µg TTHM/L) was used as the reference, OR 1.21 (95% CI: 1.09-1.35, p-trend: <0.001). No association was seen for SGA in areas using chloramine.

5.3.1.1 Preterm and very preterm delivery

When not differentiating by chlorination treatments, no association was seen for PTD newborns and second trimester TTHM exposure. When stratifying by chlorination treatment, a significant inverse association for PTD and TTHM exposure was seen in areas using hypochlorite, OR 0.90 (95% CI: 0.83–0.98, p-trend: 0.06, Table 5), comparing the population in the highest TTHM exposure (>15 µg TTHM/L) to the unexposed. The association was however not robust, as no association remained after using the <5 µg TTHM/L exposure category as the reference. No association was seen for PTD and TTHM exposure among the population in areas using chloramine.

For VPTD, a significant inverse association was observed, OR 0.82 (95% CI: 0.69–0.98, p-trend: 0.04), when combining all chlorination treatments and comparing the population in the highest TTHM exposure category to the unexposed. This inverse association did not remain in the analyses were stratified by chlorination treatment.

Table 5 Associations between trihalomethanes (TTHM; chloroform, bromoform, bromodichloromethane and dibromochloromethane) exposure and small for gestational age (SGA), preterm delivery (PTD) and very preterm delivery (VPTD), expressed as multivariable-adjusted odds ratios (OR) and 95% confidence interval (CI).

Chlorination treatment	No chlorine OR	<5 µg TTHM/l OR (95% CI)	5–15 µg TTHM/l OR (95% CI)	>15 µg TTHM/l OR (95% CI)	p-trend
Outcome: SGA					
Hypochlorite	1.00 (ref)	1.09 (0.96–1.23)	**1.14 (1.04–1.26)**	**1.20 (1.08–1.33)**	**<0.001**
		1.00 (ref)	**1.14 (1.03–1.26)**	**1.21 (1.09–1.35)**	**<0.001**
Chloramine	1.00 (ref)	0.90 (0.82–0.99)	0.94 (0.82–1.08)	0.91 (0.80–1.03)	0.4
		1.00 (ref)	1.01 (0.89–1.14)	0.96 (0.86–1.06)	0.4
Outcome: PTD					
Hypochlorite	1.00 (ref)	0.90 (0.85–0.95)	**0.85 (0.77–0.94)**	**0.90 (0.83–0.98)**	0.06
		1.00 (ref)	0.93 (0.85–1.03)	1.00 (0.93–1.07)	1.0
Chloramine	1.00 (ref)	1.04 (0.97–1.13)	1.05 (0.97–1.13)	0.95 (0.89–1.02)	0.2
		1.00 (ref)	1.04 (0.97–1.13)	0.95 (0.88–1.02)	0.2
Outcome: VPTD					
Hypochlorite	1.00 (ref)	0.95 (0.76–1.17)	0.89 (0.71–1.10)	0.88 (0.73–1.07)	0.2
		1.00 (ref)	0.99 (0.80–1.22)	0.93 (0.78–1.11)	0.4
Chloramine	1.00 (ref)	0.91 (0.78–1.06)	1.13 (0.89–1.45)	0.77 (0.62–0.96)	0.05
		1.00 (ref)	1.23 (0.99–1.52)	0.85 (0.70–1.02)	0.06

Models adjusted for maternal age, BMI, household income, attained education, smoking, country of birth, previous miscarriages, parity, sick leave/early retirement, use of teratogenic drugs, diabetes, preeclampsia, hypertension, weight gain and year of birth.

5.3.2 Congenital malformation

For all chlorination treatments, comparing the population in the highest exposed category (>15 µg TTHM/L) to the unexposed population, a significant inverse association was observed for heart defects among the newborns, multivariable-adjusted OR 0.87 (95% CI: 0.77–0.99, p-trend: 0.002). The inverse association remained for the population in areas with hypochlorite as chlorination treatment (OR 0.85, 95% CI: 0.74–0.98, p-trend: 0.03, Table 6), but not in areas with chloramine, when stratifying the analyses by chlorination treatment. When using <5 µg TTHM/L exposure category as reference, the association turned to a significant direct association in areas using chloramine, OR 1.24 (95% CI: 1.09–1.41, p-trend: 0.001).

For all chlorination treatments and comparing the population in the highest exposed category (>15 µg TTHM/L) to the unexposed, TTHM was significantly associated with malformations of the urinary system and the genitals, ORs 1.44 (95% CI: 1.10–1.89, p-trend: 0.2) and 1.47 (95% CI: 1.18–1.81, p-trend: 0.01), respectively. When stratifying by chlorination treatment, the indicated association did not remain among the population in areas

using hypochlorite, but a dose-dependent association remained for the population in areas using chloramine, corresponding to ORs 2.06 (95% CI: 1.53–2.78, p-trend: 0.001) and 1.77 (95% CI: 1.38–2.26, p-trend: <0.001) for urinary system and genitals, respectively (Table 6). For the population in areas using chloramine, but not hypochlorite, TTHM was also significantly associated with malformations of the nervous system and limbs, OR 1.82 (95% CI: 1.07–3.12, p-trend: 0.006) and OR 1.34 (95% CI: 1.10–1.64, p-trend: 0.02), respectively, comparing the highest TTHM exposure with the unexposed reference. When changing the reference category to the lowest exposed category (<5 µg TTHM/L), all dose-dependent associations remained, except for malformations of the urinary system.

No associations were observed for the development of oro-facial clefts, malformations of the digestive system or chromosomal abnormalities.

Table 6 Associations between trihalomethanes (TTHM; chloroform, bromoform, bromodichloromethane and dibromochloromethane) exposure and congenital malformations, expressed as multivariable-adjusted odds ratios (OR) and 95% confidence interval (CI).

Chlorination treatment	Non-chlorinated OR	<5 µg TTHM/l OR (95% CI)	5–15 µg TTHM/l OR (95% CI)	>15 µg TTHM/l OR (95% CI)	p-trend
Outcome: Nervous system					
Hypochlorite	1.00 (ref)	1.35 (0.75–2.45)	1.07 (0.62–1.84)	0.73 (0.39–1.37)	0.3
		1.00 (ref)	0.81 (0.44–1.50)	0.56 (0.28–1.11)	0.1
Chloramine	1.00 (ref)	0.85 (0.53–1.36)	0.89 (0.35–2.21)	**1.82 (1.07–3.12)**	**0.006**
		1.00 (ref)	0.81 (0.34–1.90)	**1.99 (1.29–3.08)**	**0.02**
Outcome: Heart Defects					
Hypochlorite	1.00 (ref)	0.91 (0.78–1.07)	0.92 (0.81–1.05)	**0.85 (0.74–0.98)**	**0.03**
		1.00 (ref)	1.02 (0.86–1.20)	0.94 (0.79–1.12)	0.5
Chloramine	1.00 (ref)	**0.75 (0.67–0.85)**	**0.74 (0.60–0.91)**	0.92 (0.79–1.06)	0.8
		1.00 (ref)	0.98 (0.80–1.20)	**1.24 (1.09–1.41)**	**0.001**
Outcome: Urinary					
Hypochlorite	1.00 (ref)	1.39 (0.99–1.94)	0.88 (0.64–1.22)	0.90 (0.65–1.26)	0.2
		1.00 (ref)	**0.64 (0.44–0.92)**	**0.65 (0.45–0.94)**	**0.02**
Chloramine	1.00 (ref)	**2.37 (1.85–3.04)**	**2.33 (1.64–3.29)**	**2.06 (1.53–2.78)**	**0.001**
		1.00 (ref)	1.06 (0.80–1.40)	0.88 (0.72–1.09)	0.3
Outcome: Genital					
Hypochlorite	1.00 (ref)	**1.36 (1.04–1.77)**	**1.30 (1.03–1.64)**	1.24 (0.97–1.58)	0.09
		1.00 (ref)	0.96 (0.74–1.25)	0.91 (0.69–1.19)	0.5
Chloramine	1.00 (ref)	1.15 (0.93–1.41)	**1.45 (1.05–1.98)**	**1.77 (1.38–2.26)**	**<0.001**
		1.00 (ref)	1.21 (0.92–1.60)	**1.50 (1.24–1.81)**	**<0.001**
Outcome: Limb					
Hypochlorite	1.00 (ref)	1.12 (0.90–1.38)	0.98 (0.82–1.19)	0.96 (0.79–1.17)	0.5
		1.00 (ref)	0.89 (0.71–1.11)	0.87 (0.70–1.10)	0.2
Chloramine	1.00 (ref)	1.06 (0.91–1.25)	1.02 (0.78–1.33)	**1.34 (1.10–1.64)**	**0.02**
		1.00 (ref)	0.96 (0.75–1.23)	**1.27 (1.08–1.49)**	**0.004**

Models were multivariable-adjusted for the following factors: maternal age, BMI (body mass index), diabetes, any use of teratogenic drugs, parity, smoking at registration to the antenatal care, highest attained education and household income using inverse probability weighting.

5.4 RISK-BENEFIT ASSESSMENT OF HYPOCHLORITE

The risk-benefit assessment for hypochlorite by using results from **Paper II-IV** are presented in Figure 8. *Scenario 1*–when the use of hypochlorite results in a high pathogen inactivation (log_{10}-redution 4.26) and low TTHM formation (<5 µg TTHM/L)–results in a reduced GII incidence among children, corresponding to a reduction in health impact of 55 DALYs per year. *Scenario 2*–when using hypochlorite for drinking water treatment results in a low pathogen inactivation (log_{10}-reduction 2.05) and high TTHM formation (>15 µg TTHM/L)–results in a lower incidence of GII among children, but also an increased risk of SGA newborns. The beneficial effects are expected to outweigh the adverse, corresponding to a reduction in health impact of three DALYs per year.

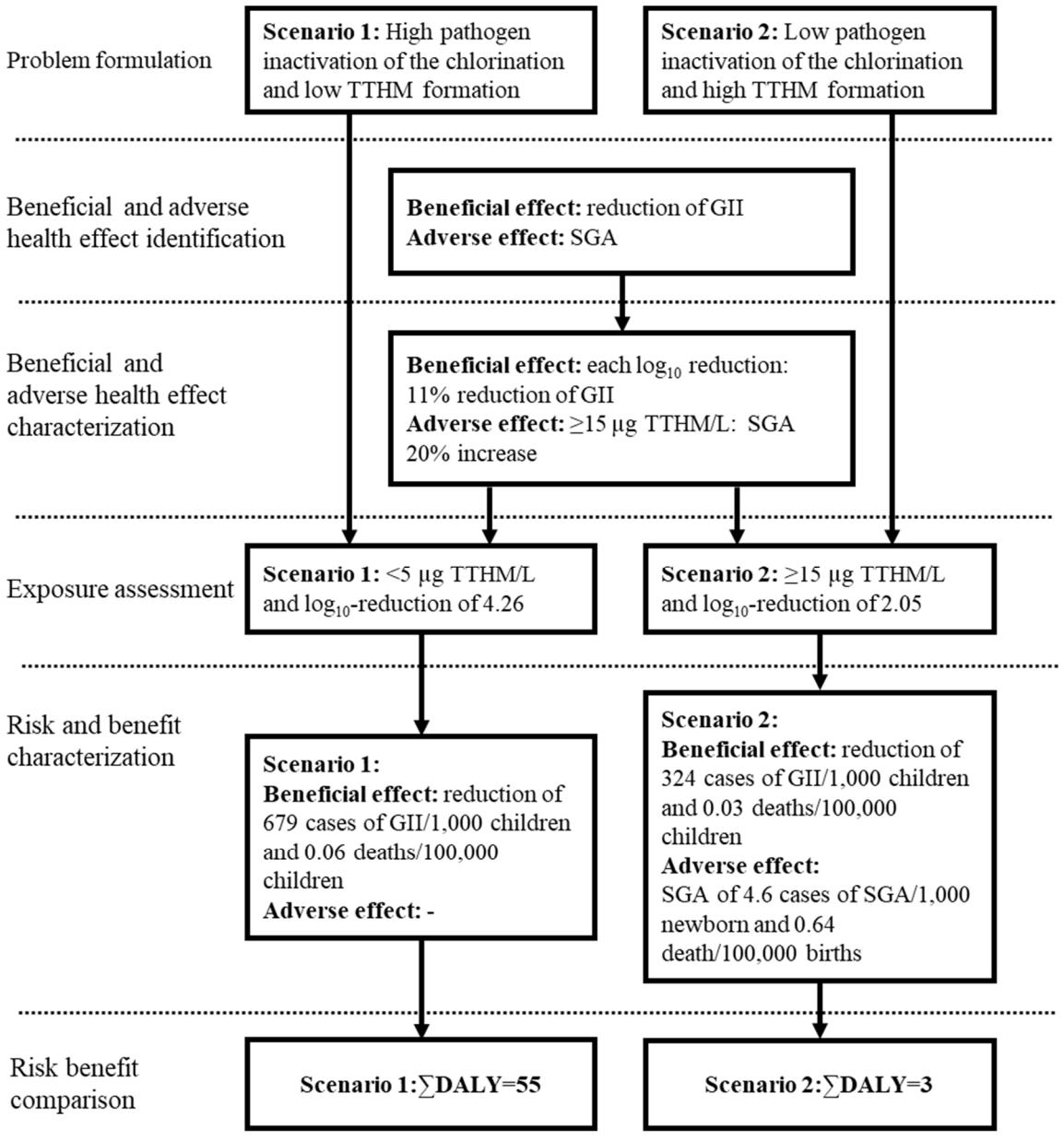

Figure 8 Preliminary risk-benefit assessment of hypochlorite in the drinking water treatment, using results from **Paper II–IV**.

6 DISCUSSION

6.1 MAIN FINDINGS

6.1.1 Summary

The main findings in **Paper I–IV**:

- From **Paper I** we can conclude that the average consumption of cold tap water among adults in Sweden was 1 L/day. Most adults (99.8%) consume cold tap water, while consumption of bottled water was low, 84% reporting to be non-consumers.
- The drinking water related changes assessed in **Paper II** did not have a significant effect on GII among the adults. However, when Partille received municipal drinking water from Gothenburg, the risk of GII among children was significantly reduced by 24% among the population receiving municipal water in Partille.
- In **Paper III**, there were indications that TTHM was significantly associated with SGA, corresponding to an OR of 1.20 (95% CI 1.08–1.33) in the population exposed to ≥15 µg TTHM/L compared to unexposed, but only in areas using hypochlorite.
- In **Paper IV**, there was indications that TTHM were significantly associated with malformations of the nervous system, urinary system, genitals and limbs, in the population in areas using chloramine. Among the newborns of mothers exposed to ≥15 µg TTHM/L in areas using chloramine, the indicated association corresponded to ORs of 1.82 (95% CI 1.07–3.12), 2.06 (95% CI 1.53–2.78), 1.77 (95% CI 1.38–2.26) and 1.34 (95% CI 1.10–1.64) for malformations of the nervous system, urinary system, genitals and limbs, respectively, as compared to newborns of unexposed mothers.
- When comparing the results from **Paper II–IV** in a risk-benefit assessment of using hypochlorite as drinking water treatment, the *preliminary* results indicate that the beneficial effects of hypochlorite overweigh the adverse, even for conditions with a high TTHM formation and low pathogen reduction.

6.1.2 Tap water consumption patterns in Sweden

6.1.2.1 In a national and international context

The average tap water consumption reported in **Paper I,** was higher than previous reported among Swedes (175-177). These differences may be due to changes in tap consumption trends over the past years or due to differences in data collection methods. In an international comparison, the tap water consumption reported in **Paper I** is close to average (178), however, some important country specific differences in consumption pattern also needs to be highlighted. In **Paper I**, and other Swedish studies, the reported tap water consumption was higher among women compared to men, opposite to what is generally reported internationally. In addition, in an international context, the reported consumption of bottled water in **Paper I** was low, with 84% of the adults reporting to be non-consumers.

6.1.2.2 Implications for ecological exposure in epidemiological research

When no individual data on drinking water consumption are available, epidemiological studies sometimes have to assume an average consumption for the entire study population, generally referred to as ecological exposure. This was the case in **Paper III-IV**, but also for the children in **Paper II**. Based on the findings in **Paper I** and data collected on children's tap water consumption, it is likely that children in **Paper II** and most pregnant women in **Paper III–IV**, will regularly consume tap water and therefore potentially be exposed to drinking water related microorganisms (**Paper II**) and CBP (**Paper III–IV**).

6.1.3 Drinking water related gastrointestinal illness

6.1.3.1 In an international context

Several epidemiological studies assessing drinking water related changes and GII, have indicated that young children constitute the most sensitive population for drinking water related GII (32, 38, 42, 43, 179, 180), thus in line with the findings reported in **Paper II**. However, it should be emphasised that in most previous studies, the change in the drinking water treatment was often initiated as a result of an initial insufficient pathogen reduction in the water treatment plant (25, 37, 181). In contrast, the changes in the treatment in **Paper II** was not mainly initiated because of suspected insufficiency in the treatment, but as a safety precaution or as a measure to secure the drinking water supply. As a result, the pathogen reduction of the water treatments were already high at baseline, which may explain why the changes of raw water and water treatment in **Paper II** did not affect the GII among adults. However, the significant risk reduction of GII among children in **Paper II** still indicates that there may be a risk of drinking water related endemic GII, especially among children, even when the water treatment has a high pathogen reduction and when the tap water has sufficient quality according to current standards.

6.1.3.2 Biological plausibility

As mentioned in the beginning of this thesis, it is well known that exposure to high levels of some pathogens may case GII. It is however unclear if the pathogen levels in the drinking water may affect population-level endemic GII. In some previous epidemiological studies on drinking water related GII, pathogen analyses were made, which support the biological plausibility of the findings seen in **Paper II**. In a study by Borchardt et al (2012), UV-light was implemented in the water treatment, significantly reducing the risk of GII, especially among children <5 years (38). In the study, monthly analyses of norovirus were made in the tap water. It was estimated that all AGI cases (self-reported, ≥3 episodes of loose watery stools or vomiting during 24h) attributable to drinking water related viruses were 6–22% and as high as 63% among children <5 years. In another study by Risebro et al. (2012), indicator bacteria (*E. coli*, Coliforms and Enterococci) were sampled in small drinking water supplies and associated with self-reported GII among the households supplied by the water (180). They observed no overall association between GII and the indicator bacteria, but for children <10 years, there was a significantly increased incidence and prevalence with the occurrence of Enterococci, with a relative risk of 4.8 for the incidence and 8.9 for the prevalence. In

conclusion, while we did not collect any biological specimen in **Paper II**, the results from previous studies point towards a biological plausibility that drinking water may be associated with GII and that children are at highest risk of developing GII.

6.1.4 Chlorination by-products and adverse reproductive outcomes

6.1.4.1 In an international context

Despite the general inconsistency in the findings from epidemiological studies, there are still some support that CBP exposure, including TTHM, may be associated with intrauterine growth retardation, like SGA (66-73), and some congenital malformations (112), thus generally in line with the findings in **Paper III**–**IV**. Still, some exposure- and outcome-related differences between **Paper III–IV** and previous studies needs to be highlighted, as these differences are relevant for the interpretations of the findings.

With a few exceptions (66, 84, 108), most previous epidemiological studies assessing CBP and SGA, have used the 10th percentile as cut-off for SGA. In **Paper III**, we used the <-2 standard deviation of the mean, which is commonly used to define SGA in Northern Europe. The difference in the case definition will affect the specificity and should therefore be considered when comparing effect estimates. In addition, studies assessing CBP and congenital malformations have suffered from lack of homogeneity for the case definition and this has been suggested to be one of the main contributors to the inconsistency in the indicated association (112).

In general, few previous epidemiological studies have reported the chlorination treatment methods used in the study areas. In **Paper III**–**IV**, we found that the indicated associations were chlorination treatment-specific, thus indicating the importance of reporting the chlorination treatment. While CBPs originating from hypochlorite (or chlorination) have commonly been assessed, based on current knowledge, no previous study has assessed CBP originating from chloramine, thus, further research is needed to confirm the findings seen in **Paper IV**.

In **Paper IV**, there were indications of an inverse association for heart defects, which have been indicated in only a few previous epidemiological studies (85, 99). The inverse association for TTHM and heart defects in **Paper IV** was however only seen in areas using hypochlorite and the association lacked robustness, as no association remained when the lowest exposed population (<5 µg TTHM/L) was assigned reference instead of the unexposed. In addition, the indicated null association turned into a direct association for the population in areas using chloramine, when assigning the population exposed to <5 µg TTHM/L as reference. Fortunately, few previous epidemiological studies have included unexposed urban reference areas, thus making it challenging to further assess these reference area-specific findings seen for heart defect. When an urban unexposed reference area was used, no indications of an inverse association for heart defects were reported (84). However, in a study using private wells, indications of inverse associations were seen for some THMs (especially brominated THMs) and heart defects (99). In conclusions, the explanation behind

the inverse association observed for the heart defects in **Paper IV** needs to be assessed in more detail.

6.1.4.2 Biological plausibility

As previously mentioned, most epidemiological studies assessing adverse reproductive outcomes used TTHM for exposure, either as the sum or individually. However, experimental studies show little support for an association between TTHM and adverse reproductive outcomes (182, 183). While some studies have indicated effects on the prenatal development, these have often been secondary to adverse effects in the dams (183). This said, there are still some indications that may support the findings seen in **Paper III-IV**.

In a few experimental studies, the primary effect has been reduced body weight and intrauterine growth restrictions among the offspring (184-187). The toxic response is suggested to be due to the formation of reactive intermediates, where the foetal metabolism is likely to play a key role (183) and cytochrome P450 2E1 gene *G1259C* has been suggested to be relevant (188). This gene is associated with increased activity of the enzyme CYP2E1 and the THM metabolism (189, 190). Still, it is important to mention that although there are some support from epidemiological studies that CYP2E1 gene *G1259C* may be important (188), later studies have yet not been able to confirm this (82, 191).

While experimental studies generally lend no support for an association between TTHM and congenital malformations, some indications of an association have been shown for other CBP (182). The strongest evidence point toward that haloacetic acids and haloacetonitriles exposure may be associated with heart defects, malformations of the limb, kidney and urogenital system (182). As craniofacial defects often have been seen together with the heart malformations in whole embryo and *in vivo* studies, neural crest cells have been suggested to play a key role in a possible mode of action (183). While the neural crest cells give rise to cells in many types of organs–like part of the nervous system, muscles, heart and limbs–this suggested pathway cannot fully explain the wide range of malformations seen in **Paper IV** (47). Another suggested mode of action is that CBP may interfere with the folic metabolic pathway (112). In a study by Dow and Green (2000), rats exposed to trichloroethylene, showed indications if B_{12}-deficiency, as methylmalonic acid increased in the urine and 5-methyltetrahydrofolate increased in the plasma (192). For trichloroethylene and other polychlorinated solvents, including chloroform (the most common THM), excess folic acid was found in the urine, not modulated by additional intake of folic acid, indicating a folate-deficiency. The authors therefore suggested that these polychlorinated solvents might interact with vitamin B_{12}, potentially through free radicals, inhibiting the methionine synthesis. Similar findings have also been seen in cell cultures (193). As the methionine synthesis is involved in the synthesis, repair and functioning of the DNA (194), it is likely that interference with the folic metabolic pathway may affect the rapid cell turnover in the organs during the foetal development. While a lack in folic acid may increase the risk of malformations of several organs, the most likely organs to be affected are the neural tube and the heart (195, 196). Still, while biological mechanism behind indicated association for congenital malformations are not yet fully understood, our chlorination treatment specific

analysis in **Paper IV** point towards a significantly increased risk for malformations of the nervous system, urinary system, genitals and limbs in areas using chloramine, but not hypochlorite. Potentially, this could be the result of a proportionally higher formation of, e.g. haloacetic acids in areas using chloramine, as compared to hypochlorite, despite similar TTHM levels (197), and that other CBP than the TTHM may be the putative agent.

6.2 METHODOLOGICAL CONSIDERATIONS

6.2.1 Study design and ethical considerations

The studies in **Paper II–IV** are all observational studies. In addition, the study presented in **Paper II** is a natural experiment. Natural experiments are not experiments, like randomized trials that follows a protocol, but instead simulates what would occur in an experiment (198). While a randomized trial is considered the gold standard for causality, not all effects of an exposure can be assessed by randomized trials, either due to practical or ethical reasons (198). The drinking water related changes in **Paper II** were not researcher-initiated and thus, does not follow the strict protocol of a regular randomized trial. Yet, the design mimics a community intervention, in which the effect of changes in the drinking water production and raw water source affects entire communities, an opportunity that rarely occurs. Moreover, as there was limited information available about when the drinking water-related changes would take place, the study had similar properties as a blinded study. Nevertheless, as the changes in the drinking water treatment were initiated by the municipal water utilities, the research question had to be set accordingly. In addition, due to several circumstances out of our control, the originally planned changes in Partille were delayed and other, unforeseen, drinking water related changes were instead captured during the study period. This resulted in a shorter data collection period than anticipated that could be used in the analyses, which affected the statistical power.

In **Paper III–IV**, we used a register-based cohort-design and tried to include as large part of the Swedish population as possible in the study, without compromising on the validity. We identified comparable densely populated urban areas around the country, of which a large part turned out to be served by non-chlorinated municipal drinking water. Due to the extensive information available in Swedish registers we obtained data linked to maternal exposure (like dates of migration and TTHM levels) and on the outcomes of the newborns. Importantly, we also obtained detailed individual information on the most important risk factors linked to the outcomes (i.e. potential confounders). This was an especially strong asset as compared to most previous large-scale studies, which often lacked information on migratory patterns and important confounders. We also managed to implement a prospective design, where the outcome, exposure and risk factors were collected independently and without interference from the researchers, as may have occurred if the data was collected by surveys or interviews.

6.2.2 Random error

Random error is when the individual measurement randomly deviates from the population average (198). These errors mainly reflect hidden factors not discovered in the study, which may affect the effect estimate. As random errors are inevitable, a confidence interval (CI) is often presented, to indicate the precision of the estimate. When the sample size in the study population increases, the random error decreases, thus the precision of the effect estimate will increase. The most common way of expressing the CI is to set it at 95%, meaning that if the study were replicated, the true effect estimate would be within the estimated CI in 95% of the replicated studies (198).

The large study population included in **Paper III–IV** will result in a low random error, as reflected by a narrow CI. In **Paper II,** we assessed the impact of drinking water related change on GII, a relative common outcome, among a large study population. However, we also assessed an interaction (baseline/follow up and exposed/reference). The assessment of an interaction requires a sample size that is several times larger than that for only assessing the main effect (199). The lower incidence of GII among adults, as compared to children, may have resulted in insufficient power to detect an effect of the GII incidence due to the implemented changes in the drinking water production.

6.2.3 Systematic error

Systematic error, commonly known as bias, is the concept of lack of internal validity or incorrect assessment of the association (198). Biases can affect the estimate in any direction: towards the null, away from the null and even change the direction of the association. While there are many types of systematic errors, a common categorization is: selection bias, information bias and confounding (200). It is crucial to reduce potential biases and have good knowledge on how inevitable biases affect the estimate.

6.2.3.1 Selection bias

Selection bias is an error introduced during the selection of study participants, when the association between the exposure and the disease is affected by factors influencing the participation (198). While selection bias may be limited by study design, the bias is often unavoidable due to factors that the researcher sometimes cannot fully control, like loss to follow-up and missing data. Multiple imputations, full-likelihood methods and IPW (also known as standardization) are examples of ways to reduce this. IPW has some advantages over other techniques when data is missing for several variables (as in **Paper III–IV**) or when there is missing data due to non-participation (as in **Paper II**) (201). As imputation models rely on the joint distribution of several variables, it can be difficult to specify a correct imputation model for a large number of categorical variables, and then IPW may be preferable. IPW is generated by including only individuals with complete data for relevant variables, i.e. exposure and confounders. Each of these individuals are then assigned a weight. By this, not only the individual herself can be accounted for, but also others not included in the data, having the same values for exposure and third variables (202).

Declining response rates have become a common challenge in epidemiological research (203) and the cohorts in **Paper I–II** are no exceptions. Participants were recruited into the study through telephone interviews and the decision to participate may be affected by several factors (study aim, presentation by the interviewer, socio-economic factors, etc.). It is relevant to highlight the low response rate, 25–44%, thus resulting in a high non-response bias. In prospective cohort studies, the participation rate may however be less of a problem as compared to case-control studies, and more often referred to as an issue of generalizability of the findings. What is important, however, is to minimize the loss to follow-up in prospective studies, as this may introduce selection bias. The study in **Paper II** lasted for several years and while we tried to encourage the participants to remain in the study, some dropouts were inevitable and the decisions to remain in the study may have introduced a selection bias. IPW was used in a sensitivity analysis of GII among children, to compensate for dropouts between two study periods. Although the IPW lead to the point estimate becoming slightly stronger, the confidence interval was widened and the association was no longer statistically significant.

In **Paper III–IV,** the probability of introducing selection bias during recruitment was low, as the health care and administrative registers included have a high coverage. Still, a selection bias may have been introduced during the stage when we assigned the exposure, as we included all newborns in the reference area, but only newborns to mothers assigned a TTHM exposure in the exposed areas. In addition, regional differences in spontaneous and induced abortion (terminated pregnancies) rates (204) may also have introduced a bias (205). For example, induced abortion due to chromosomal anomalies and multiple or single, non-chromosomal malformations were more common in certain regions of the country, such as the health care regions of Stockholm-Gotland, Western and South-Western Sweden, as compared to for example Northern Sweden. In Sweden, extensive prenatal diagnostics and screenings are available, however, there are regional differences in age groups offered screenings–such as the combined ultrasound and biochemical screening–for free (206). This could lead to regional differences in prenatal diagnosis of severe malformation (52) which potentially could affect the number of induced abortions performed. The impact of any bias linked to miscarriages and induced abortions is hard to assess, as there is no national data collected on miscarriages and the data registered on induced abortions is not possible to link to existing registers. In addition, many of the induced abortions were likely initiated based on potentially fatal congenital malformation, thus, spontaneous abortions would have occurred anyway, or could well have been initiated by other causes that were not linked to the outcome.

6.2.3.2 Information bias

Information bias arises from measurement error or a flaw in measuring the exposure, outcome or covariate variables due to differences in the quality of information between two groups (198). This misclassification generated by information bias can be either differential or non-differential, where the key variables are the exposure and the outcome. A differential misclassification refers to the misclassification differing according to values of another

variable, while for non-differential misclassification the misclassification is independent of other variables.

Exposure misclassification

As mentioned, the risk of exposure misclassification is almost inevitable when assessing CBP exposure and adverse health outcomes. Using an ecological estimate of the exposure, based on the CBP monitoring data in addition to the low TTHM formation in Swedish drinking water, there is a high risk of introducing exposure misclassification (124).

In **Paper III–IV**, we put great effort in reducing exposure misclassification, primarily by using a non-chlorinated area as the reference, stratifying the analysis by chlorination procedure, accounting for maternal migration and trying to reduce the impact of potential misclassification due to differences in municipal monitoring strategies of the TTHM. To begin with, using an unexposed reference will minimize the risk of exposure misclassification in the reference area. As mentioned earlier, unexposed reference areas have been used in some previous studies, however, in many cases, due to the general use of chlorination in the drinking water treatment, the non-exposed reference areas have often been non-urban areas with e.g. private wells, which potentially may have introduced other biases. In **Paper III–IV**, however, we were able to include an urban reference area with comparable settings as the exposed areas. Second, the stratification of the analysis by chlorination treatment made it possible to get further information on potential putative agents behind the observed associations, as there are differences in the by-products generated between the treatments. Third, in **Paper III–IV**, 16% of the women changed residence during their pregnancy, in line with what has been reported in previous studies (207). This highlights the relevance of considering migratory patterns, as this otherwise will contribute to the misclassification. Fourth, we used a trimester specific exposure, linked to the relevant effect-window for each outcome. However, due to differences in municipal monitoring of TTHM, with regional differences in monitoring strategies, we decided to use locality-specific multiannual monthly average to estimate the three-month average for each trimester.

Finally, it is important to highlight the lack of information on the exposure at the individual level, such as the drinking water consumption habits. Based on the findings from **Paper I**, we however can conclude that it is highly likely that most of the women included in **Paper III–IV** were exposed to CBP through oral exposure, as 99.8% of the adults are regular consumers of cold tap water. Yet, exposure to volatile CBP also occurs via the skin and through inhalation, thus time spent in for example the shower, also is relevant. Apparently, such information is impossible to obtain in large studies focusing on rare outcomes. This highlights the importance of including non-chlorinated areas as the reference.

Misclassification of the outcome

In **Paper II,** the outcome was based on self-defined symptoms and duration of GII. Using self-reported GII has limitations, which may affect the risk of outcome misclassification. To begin with, there was no possibility to confirm cases and to exclude non-pathogen related GII. To reduce misclassification of the outcome in **Paper II**, we used a strict case definition

for adult GII (vomiting and/or 3 loose stools during 24 h). For children, we used a pre-defined case definition. For adult, GII cases resulted in additional SMS questionnaires being sent on symptoms and duration, which may have generated a systematic bias, as some participants may have avoided reporting GII cases because of the additional workload. This bias was however not relevant for GII among children, as only one SMS question on childhood GII for the entire household was sent. In **Paper II**, we used a recall time of 28 days. Although the study had a prospective design, one may anticipate that there may be recall bias and multiple reporting of cases between the recall periods. A long recall time may introduce a bias, commonly known as telescoping, where persons remember GII episodes to be more recent than they actually were which may have affected the incidence. However, when the SMS method of collecting GII estimates was validated, the use of a 28-day recall had limited impact on the incidence (17), thus the recall bias is likely to be low.

The outcome in **Paper III–IV** originates from medical records, with high coverage and variable completeness. For **Paper III–IV**, medical personnel diagnosed the cases. While the classification of the outcomes were based on standardized protocols, misclassification may still occur due to human error or linked to factors that may lead to a biased estimate. While most of these are likely to be random errors, there are still some known systematic biases that need to be addressed (208). As mentioned earlier, gestational age is generally determined by ultrasound examinations and a systematic misclassification of the gestational age is therefore introduced among children with early foetal growth restrictions, which affect the gestational age estimate and estimates arriving from gestational age, like SGA (137). The misclassification will affect the estimate in any direction; however, if early intrauterine growth restriction (before the ultrasound examination in week 18-20) is associated with the exposure, this will result in a misclassification of PTD and an underestimation of SGA in the exposed population. In **Paper III**, we decided to include only term-SGA to reduce the risk of misclassification of the outcome. As SGA is based on weight-for-gestational-age charts for those that have been born, a "missing data" problem is generated, as no weight data will be available for babies that still are in the uterus (i.e. if not restricted to term-SGA). This will lead to a case definition that is inconsistent across gestational ages and thus a biased SGA estimate among preterm delivered newborns, but not full-term (209). Although there have been suggestions on how to deal with this issue, no study has yet appropriately addressed a solution to this problem with current weight-for-gestational-age charts, especially as the missing data is not at random (209). While using term-SGA as in **Paper III** may solve some issues, one should be aware that conditioning on the case definition i.e. in this case on term/preterm delivery might introduce a biased pathway through a potential unmeasured confounder (Figure 9), which may result in a biased estimate (collider stratification bias).

As congenital malformations are rare, we used major congenital malformations for categorization of the outcome in **Paper IV**. As mentioned earlier, this may have led to the outcomes being pooled together, despite potential differences in the underlying biological mechanism. Classification of congenital malformations is challenging, especially for those resulting from errors of morphogenesis (47). Moreover, the biological mechanisms behind malformations are generally not well understood, making accurate categorization impossible.

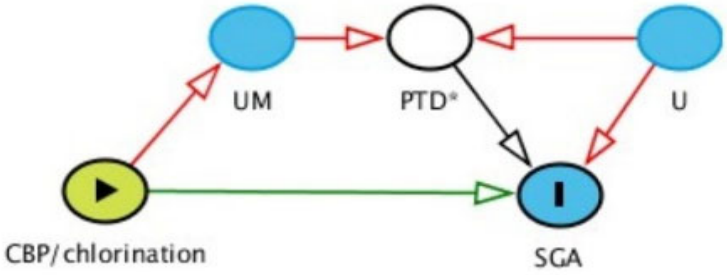

Figure 9 Directed acyclic graph (DAG) for the association between chlorination by-product (CBP) exposure and small for gestational age (SGA), if conditioned on term/preterm delivery (PTD) in the presence of a common unmeasured confounding (U) for PTD and SGA. UM=unknown mechanism, *=conditioned on, red arrow=bias pathway, green arrow=causal path, green circle=exposure, blue circle=outcome or ancestor to outcome, hollow circle=variable conditioned on.

We only obtained information on congenital malformations from the Medical Birth Register, which includes malformations registered up to 28 days postpartum. While most severe congenital malformations are discovered by this period, some less severe malformations may be discovered later in life (204). In Sweden, it is possible to obtain additional data on congenital malformation from inpatient and outpatient care, included in the Patient register (administered by the National Board of Health and Welfare). Although, we initially planned to include diagnosis obtained from these additional registers throughout age five of the included children, we decided against that during the course of the project, due to uncertainties of the quality/validity, especially for the diagnosis obtained from the outpatient care (based on information from the National Board of Health and Welfare).

6.2.3.3 Confounding

Confounding refers to a confusion of effects, meaning that the effect of the exposure-outcome association is being mixed with the effect of another factor (198). The presence of a confounder may mask or falsely demonstrate an exposure-outcome association. Confounders may affect the true effect in any direction and even alter the direction of the effect. While confounders have a clear definition, selecting confounders is not straightforward. As suggested by Rothman et al., a confounder must fulfil three criteria (198). First, a confounder must be an extraneous risk factor of the disease, meaning that the association with the confounder and the outcome arises from a different causal pathway than the one under study. While some confounders are risk factors for the outcome, a confounder can also be a surrogate of a risk factor. Second, a confounder must be associated with the exposure under study in the source population. In cohort studies, the confounder-exposure association can be determined by the study data. However, the effect of a confounder is always conditioned on other factors being controlled for (198). This means that each new stratum generated by adjusting for a new confounder, will affect the confounder-exposure association of all other confounders included. Third, and importantly, a confounding factor must not be affected by (i.e. a result of) the exposure or the disease. This is includes if the confounder is in the causal pathway, thus being an intermediating factor between the exposure and the disease.

Adjustment of an intermediator results in an over-adjustment bias (i.e. not observing the full effect), often resulting in the total effect moving towards the null (210, 211).

A limitation in **Paper II** was the lack of information on several potential confounders. While we have been able to consider the most relevant confounders, like attendance at day-care for children, there is still limited knowledge of non-drinking water related causal pathways, especially person-to-person transmission. However, due to the nature of the infectious diseases, it is hard to identify representative confounders, especially for non-epidemic diseases. It should also be highlighted that infectious diseases have some special features not relevant for non-infectious diseases (212). The two most important ones are that a case may be a risk factor and that persons in the study population may develop immunity. Making things even harder, a person can be a risk factor, even without being a case, if the person is an asymptomatic carrier. In **Paper II**, we included age, gender, having children age 0–5 years and water consumption in the adult model, but only children at day-care in the model for 0–9 year-olds. While we were unable to collect information on all relevant confounders, high emphasis was still put on assessing factors on a population-level, that may have affected the GII prevalence. To begin with, we checked nationwide statistics of norovirus infection, to assess the interannual differences, which may have affected the possibility to compare years. While other infections than norovirus may be relevant, current knowledge on GII in Sweden indicate that the majority of GII cases are likely to be of viral origin (17), thus norovirus infections may give a fair estimate of interannual differences in GII incidence. The results showed little difference in norovirus infections between the years of data collection. During the end of the data collection period, rotavirus vaccination of infants was included in the vaccination program in one of the areas. While this may have contributed to regional differences in the GII incidence, the risk of affecting the outcome was low, as the vaccination program was implemented at the end of the study and during a time of the season with a generally low rotavirus prevalence. We also looked at the rate of water pipe breaks in the areas and found that the rate was similar between the study periods and areas.

While several risk factors have been established for the outcomes under study in **Paper III**, fewer have been identified for congenital malformation in **Paper IV**. As the most relevant confounders had little impact on the effect estimate, it is unlikely that additional confounders would have affected the indicated exposure-outcome associations to a significant extent in **Paper IV**. It is also well known that epigenetics, the genotype of the mother and child, may play a key role in the biological mechanisms behind the development of malformation (47), thus it is likely that there may be effect modifiers that we were not able to consider in the analysis, as a result of the study design. Still, as highlighted earlier in the thesis, the knowledge on the impact of epigenetics and genotype of mothers and children on the development of congenital malformations is still limited.

6.2.4 External validity

While internal validity is to make accurate assessment of the association, external validity refers to the generalizability of the results to other populations. The external validity is highly

dependent on internal validity, although additional factors may also be relevant for the generalizability of the results (200).

In **Paper I–II,** high emphasis was put on finding a representative sample of the population in the study area and to confirm that the findings were representative of the general population, i.e. several nation-wide surveys were conducted in parallel to the study population to confirm the findings. The study area in **Paper II** was limited to the area where the planned drinking water related changes were expected. Still, the raw water conditions and water treatment used in the study areas are representative for the drinking water related conditions in Sweden. In **Paper III–IV**, we included all newborns in the study area and during the selected study period, thus making it a nation-wide study. While there may be differences between the Swedish population and other populations, in terms for example unmeasured confounders the results from **Paper III–IV** still most likely have a high generalizability.

7 CONCLUSIONS

Based on the results from **Paper I–IV,** it can be concluded that

- Almost all adult Swedes consume cold tap water on a regular basis, implying that any potential microbiological or chemical contamination of drinking water has the ability to reach and affect substantial parts of the population. It also lends support to the use of large register-based studies in research on drinking water quality and health.
- Change in drinking water treatment, that increase pathogen reduction from an already sufficient quality–according to current standards–may still reduce the risk of gastrointestinal illness among children.
- Despite generally low average concentrations of four estimated chlorination by-products present in the residential drinking water of pregnant women in Sweden, dose-dependent associations were observed with increased risk of being born small for gestational age (restricted to hypochlorite treatment) and with certain malformations (restricted to chloramine treatment). The findings are a matter of concern, but need to be interpreted with caution, warranting confirmation by other studies. Nevertheless, although challenging, it is important that drinking water producers continuously strive towards providing tap water with as low chlorination by-product levels as possible.

8 POINTS OF PERSPECTIVES

- Further research is needed to assess drinking water related endemic gastrointestinal illness, especially among children, and to understand the link between pathogen-specific reduction in the water treatment and gastrointestinal illness. In future studies, special attention should be paied to improving the specificity of the outcome, e.g. by including measurements of pathogens in drinking water and faeces among the participants and/or to use a more strict case definition.
- Considering the chlorination treatment-specific associations observed for TTHM and several adverse reproductive outcomes in **Paper III-IV**, more attention on this issue is clearly needed. This includes improved knowledge on the different chlorination by-products actually generated by the two treatments and to assess the putative agent(s) behind the observed treatment-specific associations. In addition, despite the lack of statistical robustness, the explanation for the inverse association observed for the heart defects in **Paper IV** needs to be scrutinized in more detail, considering that similar findings have been indicated in other epidemiological studies.
- Based on the combined results from **Paper II-IV**, a preliminary risk-benefit assessment of using hypochlorite in the drinking water treatment was performed, indicating that the beneficial effects of hypochlorite seems to outweigh the adverse effects at the TTHM concentrations detected in Sweden (**Appendix 1**). However, the findings should be interpreted with great caution, as data from additional studies are needed to fully consider all potential short- and long-term health consequences and to reduce current uncertainties. Undoubtedly, risk-benefit assessment is a tool that can be used in the future to support decision making regarding drinking water treatment.

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10 APPENDIX

10.1 APPENDIX 1

PRELIMINARY RISK-BENEFIT ASSESSMENT OF DRINKING WATER HYPOCHLORITE–AN EXAMPLE OF USING RESULTS FROM PAPER II-IV FOR DECISION SUPPORT

Introduction

The findings from **Paper II-IV** are implemented in a quantitative risk-benefit assessment of hypochlorite (common chlorination treatment), according to the procedure described by EFSA (127). As only data from **Paper II-IV** are considered, this risk-benefit assessment only illustrates an example and the results should be interpreted with caution.

Problem formulation

This risk-benefit assessment of drinking water hypochlorite is restricted to epidemiological results presented in **Paper II-IV**. Focusing on Swedish conditions and both ends of the extreme conditions during normal operation, the following scenarios will be assessed:

Scenario 1: High pathogen inactivation by hypochlorite and low TTHM formation

Scenario 2: Low pathogen inactivation by hypochlorite and high TTHM formation

Scenario 1 is a situation where a raw water is low in organic matter and other naturally occurring substances–which may generate TTHM–and where the pathogen reduction is assumed to be as efficient as possible (*e.g.* ground water or chlorination after slow sand filtered low turbid surface water). *Scenario 2* is a situation with a high water content of substances that may generate a high TTHM formation, corresponding to a low pathogen reduction (*e.g.* turbid surface water). The pathogen inactivation (log_{10}-reduction) and TTHM levels is quantified under the 'Exposure assessment' section.

Beneficial and adverse health effect identification

Beneficial effect: The reduction of GII among children related to drinking water hypochlorite, estimated based on the risk reduction of GII reported among children due to changes in the drinking water treatment and raw water source reported in **Paper II**.

Adverse effects*:* The indicated significant associations for gestational TTHM exposure and adverse reproductive outcomes (SGA) as reported for newborns in areas using hypochlorite in **Paper III**. No indication of an association for TTHM and adverse reproductive outcomes were seen for hypochlorite in **Paper IV**.

Beneficial and adverse health effect characterization

Beneficial effect: The beneficial effects of drinking water hypochlorite will be quantified by estimating the dose-response for GII and the pathogen reduction based on the drinking water related changes in **Paper II.** As the water utilities in Sweden generally have a high pathogen reduction, we can assume the pathogen exposure to be low, thus a linear extrapolation is recommended and can be used for the dose-response (169). The slope (*m*) of this log-linear dose-response (based on data from **Paper II)** will be used to calculate the yearly reduction of GII cases from using hypochlorite. The calculations are presented later in the Appendix, but will be presented in short here.

When estimating the exposure in **Paper II**, two aspects have to be considered: *i)* change in pathogen $\log_{10}$-reduction and *ii)* change in pathogen levels. Pathogen inactivation will be quantified as the weighed theoretical $\log_{10}$-reduction ((36), with information from the water utilities). The method is presented by Tornevi et al. (36), and is based on the mean value of the $\log_{10}$-reductions for the water treatment for bacteria, virus and parasites, where each pathogen group is weighted based on how commonly these pathogen groups cause GII (pathogen weights (168): virus=0.79, bacteria=0.05, protozoa=0.16). The total $\log_{10}$-reduction of each treatment step is based on the mean elimination capacity (5), where values represent the central tendencies between lowest and highest values. Based on this method, the estimated weighted $\log_{10}$-reduction was 3.5 in Partille and 7.5 in Gothenburg, corresponding to a difference in $\log_{10}$-reduction of 4.0 between the two treatment plants (Table 7). As reported in **Paper II**, the occurrence of the bacteria *Escherichia coli* (as the average pathogen load, colony-forming units [CFU]/100ml) during 2000-2011 was about 70 times higher in the river used for raw water intake in Gothenburg, compared to the lake used for raw water intake in Partille, corresponding to a $\log_{10}$-reduction of 1.8. Based on the difference in the pathogen load in $\log_{10}$-units (i.e. $\Delta\log_{10}$Patogen=1.8) and the difference in the $\log_{10}$-reduction between the two water treatment plants ($\Delta\log_{10}$reduction=4.0)–assumed to reflect a change in exposure–the estimated theoretical change in $\log_{10}$-reduction was estimated to be 2.2 ($\Delta\log_{10}$ reduction-$\Delta\log_{10}$Patogen).

Table 7 Estimated theoretical $\log_{10}$ reduction in viruses, bacteria and protozoa, and the weighted $\log_{10}$-reduction.

		∑log₁₀-reduction (mean elimination capacity)			
Municipality	**Microbiological barriers**	**Viruses**	**Bacteria**	**Protozoa**	**Weighted theoretical log₁₀-reduction**
Partille	chlorination and ozonation	3.4	15	0.5	3.5
Gothenburg	chlorination, UV disinfection, flocculation followed by disc filters	7.3	11	7.3	7.5

Mean elimination capacity (5), where values represent the central tendencies from interval lowest to highest values. **Weighted theoretical $\log_{10}$-reduction** (168): virus=0.79, bacteria=0.05, protozoa=0.16.

When estimating the associated change in incidence of infection the results in **Paper II** indicated no effect on the GII incidence among adults due to drinking water related changes, but a 24% risk reduction of vomiting and/or diarrhoea for children receiving drinking water from the neighbouring municipality Change 3b). The estimated theoretical change in log_{10}-reduction of 2.2, would correspond to an 11% reduction in the risk of vomiting and/or diarrhoea among children for each log_{10}, corresponding to a log-linear dose-response trend with a slope of 0.11.

Adverse effect: In **Paper III,** we observed indications of a dose-dependent association for TTHM exposure and SGA in areas using hypochlorite. The odds of being born SGA was 20% higher, comparing the highest exposure group (>15 µg TTHM/L) to the unexposed. We observed no indications of a direct association for TTHM exposure and preterm delivery (**Paper III**) or congenital malformations (**Paper IV**) in areas using hypochlorite.

Exposure assessment

Beneficial effect: The lowest and highest weighed theoretical log_{10}-reduction for hypochlorite are estimated to be 2.05 and 4.26, respectively (Estimated log_{10}-redution for hypochlorite: virus 2.5-5.0, bacteria 1.5-3.0, protozoa 0-1.0; Estimated pathogen weights: virus=0.79, bacteria=0.05, protozoa=0.16) (5, 168).

Adverse effect: Based on the exposure in **Paper III**, <5 µg TTHM/L and >15 µg TTHM/L will be used as exposures corresponding to a low and high TTHM exposure scenario, respectively.

Exposure assessment by scenario:

Scenario 1: <5 µg TTHM/L and theoretical pathogen log_{10}-reduction of 4.26 when hypochlorite is used.

Scenario 2: >15 µg TTHM/L and theoretical pathogen log_{10}-reduction of 2.05 when hypochlorite is used.

Risk and benefit characterization

Scenario 1:

Beneficial effect: Based on the exposure resulting in a log_{10}-reduction of 4.26, each log_{10}-reduction units corresponds to an 11% decrease in vomiting and/or diarrhoea among children. With a yearly incidence of 1.45 vomiting and/or diarrhoea among children 0-9 years (**Paper II**), we estimate that chlorination with hypochlorite would result in a yearly risk-reduction of 679 cases of vomiting and/or diarrhoea/1,000 children. Yearly GII-related deaths rate among 0-9 year olds is estimated to be 0.14 deaths/100,000 children 0-9 years (Table 8). Assuming the drinking water related cases of GII could be fatal, an 11% decrease in the GII cases

among 0-9 year, would result in the reduction of 0.015 deaths/100,000 children age 0-9 years for each $\log_{10}$-reduction unit. For *Scenario 1*, with a high pathogen reduction, the number of reduced yearly deaths would be 0.06 deaths/100,000 children and year.

Adverse effect: No adverse effect.

Scenario 2:

Beneficial effect: Based on the exposure resulting in a $\log_{10}$-reduction of 2.05, and assuming the same conditions as for *Scenario 1*, we estimate that chlorination with hypochlorite would result in a yearly risk-reduction of 324 cases of vomiting and/or diarrhoea/1,000 children. Assuming the same conditions for GII related deaths for *Scenario 1*, *Scenario 2* would result in the yearly reduction of 0.03 deaths/100,000 children aged 0-9 years.

Adverse effect: the adverse effect corresponds to a 20% increase in the risk of SGA, as reported in **Paper III**. In Sweden, the yearly incidence of SGA during 2005-2015 was 23 cases of SGA/1,000 newborns (4), thus *Scenario 2* results in an increase in SGA of 4.6 cases of SGA/1,000 newborns. Yearly deaths linked to low birth weight (ICD10: P07, 2005-2015) is 3.2 deaths/100,000 births (Table 8). Assuming that low birth weight and SGA are equivalent in terms of deaths, a 20% increase in the risk of SGA would correspond to an increase of 0.64 death/100,000 births in *Scenario 2*.

Risk-benefit comparison

Quantifying the Burden of disease from mortality and morbidity

DALY is estimated by the sum of Years Lived with Disability (YLD), which holds information on severity, incidence and duration, and Years of Life Lost (YLL), which is linked to premature death:

$$DALY=YLL+YLD$$

$$YLL=N \cdot L$$

where N is the number of deaths and L is the standard life expectancy at age of death in years, and

$$YLD=P \cdot D \cdot DW$$

where P is the number of cases, D is the duration and DW is disability weight.

Selecting disability weight and duration of disease

Beneficial effects: As most cases of GII among children are mild in Sweden, it is most appropriate to use a disability weight for mild GII and with a short duration. The most commonly used disability weight for mild diarrhoeal diseases have been 0.074 for each

episode (213-215). The duration of GII is also short, for the adult Swedish population it is estimated to be 2.3 days (17), thus the appropriate duration of disease would be 0.01 years per episode, corresponding to 3.5 days, in line with other estimates used for duration of GII (174).

Adverse effects: Despite a low weight at birth, majority of the children born SGA will accelerate in growth already during early childhood (56). SGA is also associated with long-term health effects, like cardiovascular and metabolic diseases and chronic hypertension (57-60) and may affect the intellectual and educational outcome (61). However, these long-term effects will not be considered in the present risk-benefit assessment, as this was not assessed in **Paper II-IV**.

Disability weights have been suggested for low birth weight and these are considered applicable for SGA. A disability weight of 0.442 will be used for SGA, with a duration of one year (174), in line with other disability weights suggested for low birth weight (173).

Life expectancy, number of deaths and relevant population size

We use the Swedish estimates for life expectancy for women at year 2060, thus 89.1 years (172). The number of deaths for each outcome is presented in Table 4.

For comparison of estimates, we use the nationwide estimates of the population, thus having a yearly average population of 100,000 children 0-9 years and 10,000 newborn. This is estimated to be the population in Sweden potentially exposed to >15 µg TTHM/L from hypochlorite during one year.

Table 8 Parameters included in the estimation of the risk-benefit assessment

Parameter	Estimate	Explanation	Source or equation
Adverse effects: increased risk of SGA			
SGA_{TTHM}	20%	Increase in the risk of giving birth to a SGA infant among mothers exposed to >15 µg TTHM/L	Paper III
I_{SGA}	23 cases of SGA/1,000 newborn	yearly incidence of SGA	Paper III
$I_{SGATTHM}$	4.6 cases of SGA/1,000 newborn	Estimated increase in the risk of SGA among mothers exposed to >15 µg TTHM/L	$SGA_{TTHM} \cdot I_{SGA}$

Parameter	Estimate	Explanation	Source or equation
N_{SGA}	3.2 children/ 100,000 newborn and year	Yearly deaths linked to low birth weight (ICD10: P07, Sweden during 2005-2015)	(170, 171)
$N_{SGATTHM}$	0.64 deaths/100,000 newborn and year*	Yearly increase in low birth/SGA-related deaths of individuals with mothers exposed to TTHM levels >15 µg TTHM/L	$N_{SGA} \cdot SGA_{TTHM}$
Beneficial effects: reduced GII			
$Log10_P$	3.5	Estimated theoretical weighted log_{10}-reduction of the treatment in Partille	(5, 36, 168)
$Log10_G$	7.5	Estimated theoretical weighted log_{10}-reduction of the treatment in Gothenburg	(5, 36, 168)
$\Delta log_{10} reduction$	4	Estimated theoretical weighted log_{10}-reduction during Change 3b	(Log10G-Log10P
$\Delta logPatogen$	1.8	Estimated log_{10}-difference in the pathogen load between Gothenburg and Partille during the period, i.e. 70 times ($10^{1.8}$) higher in the river in Gothenburg compared to the lake in Partille	Paper II
$\Delta log10$	2.2	Estimated theoretical change in log_{10}-reduction of the water treatment between Partille and Gothenburg in Paper II, considering the difference in pathogen load between the raw water sources. This estimate is assuming a liner low dose extrapolation on a log/log scale	Δlog10reduction-ΔlogPatogen
ΔGII	(-)24%	Reduction of vomiting and/or diarrhoea among children in Partille in Paper II	Paper II
m	-0.11	Estimated slope for the linear trend between the GII incidence and the theoretical log_{10}-reduction in the water treatment, based on data from Paper II. The slope corresponds to 11% reduction of GII per log_{10}-unit	ΔGII/Δlog10
I_{GII}	1,450 cases of GII/1,000 child and year	Yearly incidence of vomiting and/or diarrhoea among children 0-9 years	Paper II
$Log10_{Low}$	2.03	Estimated theoretical log_{10}-reduction of hypochlorite for scenario with a low pathogen reduction	(5)

Parameter	Estimate	Explanation	Source or equation
$Log10_{High}$	4.26	Estimated theoretical log_{10}-reduction of hypochlorite for scenario with a high pathogen reduction	(5)
I_{GIILow}	324 cases of GII/1,000 children and year	Estimated yearly reduction of GII among children 0-9 years in a scenario with a low log_{10}-reduction of hypochlorite	$m \cdot I_{GII} \cdot Log10_{Low}$
$I_{GIIHigh}$	679 cases of GII/1,000 children and year	Estimated yearly reduction of GII among children 0-9 years in a scenario with a high log_{10}-pathogen reduction of hypochlorite	$m \cdot I_{GII} \cdot Log10_{High}$
N_{GII}	0.14 deaths/100,000 children and year	Yearly incidence GII-related deaths among 0-9 year old in Sweden (ICD10: A00-A10, year 2010-2019)	(170, 171)
$N_{GIIlog10}$	0.015 deaths/100,000 children and year	Yearly GII-related deaths among children 0-9 years in a scenario with a low log_{10}-pathogen reduction of hypochlorite	$N_{GII} \cdot \Delta logPatogen$
N_{GIIlow}	0.03 deaths/100,000 children and year	Yearly reduction in GII-related deaths among children 0-9 years in a scenario with a low log_{10}-reduction of hypochlorite	$N_{GIIlog10} \cdot Log10_{Low}$
$N_{GIIHigh}$	0.06 deaths/100,000 children and year	Yearly reduction in GII-related deaths among children 0-9 years in a scenario with a high log_{10}-reduction of hypochlorite	$N_{GIIlog10} \cdot Log10_{High}$

*assuming a similar death rate due to SGA as low birth rate

Uncertainties

Beneficial effects: When estimating the beneficial effects, several assumptions contribute to a high uncertainty of the estimate, including the assumed linear dose-response, average *E. coli* in the raw water and how this correlate to the actual pathogen load, as well as the average theoretical pathogen log_{10}-reduction in the treatment and the estimated weights for log_{10}-reduction. In addition, the data and the results in **Paper II** come with several uncertainties, like the lack of knowledge on relevant risk factors, the lack of being able confirm GII cases, the use of municipal monitoring data to estimate the differences in pathogens in the raw water, and the lack of information on actual pathogen reduction as a result of change in water treatment. The use of an ecological exposure for the tap water intake among children, may also introduce uncertainties, especially when the results are extrapolated to other populations. In conclusion, the beneficial effect estimates are therefore likely to be uncertain.

Adverse effects: **Paper III** is a nation-wide study, including all newborns in the study area and during the study period living in areas relevant for the exposure. The study also had a

high coverage of the register included and extensive information on risk factors. The ecological exposure–i.e. the lack of data on tap water consumption and other routes of exposure–and the risk of exposure misclassification, may have introduced uncertainties into the estimate. Still, a large study population and the use of a non-chlorinated reference area will reduce the impact of any exposure misclassification considerably. In conclusion, the adverse health effects estimate therefore is likely to be less uncertain.

Results and conclusions

For *Scenario 1*, drinking water treated with hypochlorite will have only beneficial effects, resulting a reduced risk of GII among children, thus resulting in the reduction of 55 DALYs, because of reduced GII among children 0-9 years (Table 9). For *Scenario 2*, both adverse and beneficial effects are expected, but with a higher impact of the beneficial health effects, resulting in the reduction of 3 DALYs.

In conclusion, according to the scenarios evaluated, drinking water treated with hypochlorite will result in the beneficial effects outweighing the adverse effects for Swedish conditions. As high TTHM levels comes with adverse reproductive outcomes, drinking water producers should continuously strive towards providing tap water with as low chlorination by-product levels as possible. Still, due to uncertainties (especially linked to the beneficial effects) and only including data from **Paper II-IV**, and as no long-term health effects are considered, these results should be seen as illustrative for the potential of using risk-benefit assessments for decision support and at this stage should be interpreted with caution.

Table 9 Estimation of disability-Adjusted Life Years (DALY) for *Scenario 1* and *2*, estimating a population size of 10,000 births and 100,000 children age 0-9

Outcome	N	L	YLL	P	D	DW	YLD	DALY
Scenario 1								
Beneficial effect: GII	0.06	89.1	5	67,900	0.01	0.074	50	55
Adverse effect: -								
∑DALY								55
Scenario 2								
Beneficial effect: GII	0.03	89.1	3	32,400	0.01	0.074	24	27
Adverse effect: SGA	0.064	89.1	6	46	1	0.4	18	24
∑DALY								3

N= number of deaths, L= life expectancy at age of death in years, YLL= Years of Life Lost, P= number of cases, D=duration of disease in years, DW=disability weight, YLD=Years Lived with Disability, GII=gastrointestinal illness 0-9 years, SGA= small for gestational age

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