

Health impacts of long-term exposure to disinfection by-products in drinking water in Europe: HIWATE

Mark J. Nieuwenhuijsen, Rachel Smith, Spyros Golfinopoulos, Nicky Best, James Bennett, Gabriella Aggazzotti, Elena Righi, Guglielmina Fantuzzi, Luca Bucchini, Sylvaine Cordier, Cristina M. Villanueva, Victor Moreno, Carlo La Vecchia, Cristina Bosetti, Terttu Vartiainen, Radu Rautiu, Mireille Toledano, Nina Iszatt, Regina Grazuleviciene and Manolis Kogevinas

ABSTRACT

There appears to be very good epidemiological evidence for a relationship between chlorination by-products, as measured by trihalomethanes (THMs), in drinking water and bladder cancer, but the evidence for other cancers, including colorectal cancer appears to be inconclusive and inconsistent. There appears to be some evidence for a relationship between chlorination by-products, as measured by THMs, and small for gestational age (SGA)/intrauterine growth retardation (IUGR) and preterm delivery, but evidence for other outcomes such as low birth weight (LBW), stillbirth, congenital anomalies and semen quality appears to be inconclusive and inconsistent.

The overall aim of the HIWATE study is to investigate potential human health risks (e.g. bladder and colorectal cancer, premature births, SGA, semen quality, stillbirth, congenital anomalies) associated with long-term exposure to low levels of disinfectants (such as chlorine) and DBPs occurring in water for human consumption and use in the food industry. The study will comprise risk–benefit analyses including quantitative assessments of risk associated with microbial contamination of drinking water versus chemical risk and will compare alternative treatment options. The outcome will be improved risk assessment and better information for risk management. The work is divided into different topics (exposure assessment, epidemiology, risk assessment and management) and studies.

Key words | cancer, chlorination, disinfection by-products, epidemiology, reproductive health, risk assessment

Mark J. Nieuwenhuijsen (corresponding author)

Cristina M. Villanueva

Manolis Kogevinas

Centre for Research in Environmental

Epidemiology (CREAL), Parc de Recerca

Biomèdica de Barcelona-PRBB (office 183.05),

C. Doctor Aiguader, 88, 08003 Barcelona,

Spain

Tel.: (+34) 93 316 0646

Fax: (+34) 93 316 05 75

E-mail: mnieuwenhuijsen@imim.es

Municipal Institute of Medical Research

(IMIM-Hospital del Mar) and CIBER

Epidemiologia y Salud Pública (CIBERESP),

Spain

Mark J. Nieuwenhuijsen

Rachel Smith

Nina Iszatt

Nicky Best

James Bennett

Mireille Toledano

Imperial College London, UK

Spyros Golfinopoulos

University of the Aegean, Greece

Terttu Vartiainen

National Public Health Institute, Finland

Manolis Kogevinas

University of Crete, Greece

Sylvaine Cordier

Université de Rennes, France

Gabriella Aggazzotti

Elena Righi

Guglielmina Fantuzzi

University of Modena and Reggio, Italy

Radu Rautiu

ICON Ltd, UK

Regina Grazuleviciene

Vytautas Magnus University, Lithuania

Carlo La Vecchia

Cristina Bosetti

Istituto di Ricerche Farmacologiche 'Mario Negri',

Italy

Luca Bucchini

Hylobates Consulting Srl, Italy

Victor Moreno

Catalan Cancer Institute (ICO), Spain

INTRODUCTION

It has been more than 30 years since trihalomethanes (THMs) were first discovered in the Netherlands (Rooks 1974). Chlorination disinfection by-products (DBPs) are formed when water is chlorinated and the organic matter in the water reacts with chlorine to form these by-products. The formation and occurrence depends on many factors, including the chlorine dose, type of treatment, pH, temperature, residence time and bromine levels (Nieuwenhuijsen *et al.* 2000a; IPCS 2000). Up to 600 DBPs have been identified (Richardson 1998; Richardson *et al.* 2008). Different mixtures of by-product may exist in different locations depending on the various factors mentioned above, making it more difficult to assess any health effects of DBPs, particularly in epidemiological studies. In Europe there is relatively little known about the occurrence of DBPs other than THMs and their levels (Palacios *et al.* 2000), with some exceptions in a few places such as Poland (Dojlido *et al.* 1999), Finland (Nissinen *et al.* 2002), Spain (Villanueva *et al.* 2003), the UK (Malliarou *et al.* 2005), Greece (Golfinopoulos & Nikolaou 2005) and Italy (Fantuzzi *et al.* 2007), while in the US extensive surveys have been conducted to assess DBP occurrence under different water treatment methods (e.g. Weinberg *et al.* 2002; Krasner *et al.* 2006).

In the USA and Canada there has been considerable progress in the assessment of health risks and policy development in relation to DBPs, including a research programme on the occurrence and health risks relation to the by-products by the USEPA's office on Water and National Health and Environmental Effects Research Laboratory (http://www.epa.gov/nheerl/research/drinking_water.html). A considerable amount of work has been carried out measuring a range of different DBPs, animal testing of a list of high priority by-products and epidemiological studies. However, results may not be extended to Europe because mixtures of DBPs may be different as a result of different determinants such as treatment, total organic content (TOC), pH etc. In Europe there has been a much slower response to the recent findings. Disinfection is used in many countries in Europe and is therefore of European concern and requests a European approach and solution. Relatively little research has been carried out on DBPs in relation to adverse birth

outcomes and cancer in Europe. Where work has been carried out, this has been carried out in isolation.

Safe drinking water has a high priority in Europe in accordance with the Water Framework Directive and the Directive on Quality of Water intended for Human Consumption. Water treatment safety has become particularly acute since the quality of water resources may be declining because of water scarcity in some regions, increasing the cost of drinking water production and the likelihood of chemical interactions during the treatment process. Water is an important part of the food chain. Consumer health and well-being, quality, safety and consumer concern, are highly important and should be addressed where possible, particularly where environmental health risks are concerned. Recently there has been consumer concern about the quality of drinking water from the tap and this may have led to an increase in the consumption of expensive bottled water in developed nations (Doria *et al.* 2005; Doria 2006), reducing the money that can be spent on more beneficial items.

Ingestion of water may not be the only concern since an individual can also be exposed to volatile DBPs (e.g. THMs) through inhalation and absorption, during activities such as showering, bathing and swimming (Nieuwenhuijsen *et al.* 2000a). Recent modelling has suggested that this route may lead to the highest levels in the blood (Whitaker *et al.* 2003). Uptake through showering, bathing and swimming showed considerable increased risk in a recent bladder cancer study (Villanueva *et al.* 2007). For non-volatile DBPs, such as haloacetic acids (HAAs), ingestion is thought to be the main route of exposure.

In this paper we briefly summarise the epidemiological evidence and limitations regarding the two main areas of health effects from exposure to DBPs, cancer and reproductive effects. We then present the background and design of a major research initiative in the EU (Health Impacts of long-term exposure to disinfection by-products in drinking WATER, HIWATE project) that will provide an extensive evaluation of exposure, hazard identification, risk assessment and risk benefit analysis for these compounds in the EU.

EPIDEMIOLOGICAL STUDIES EXAMINING HEALTH EFFECTS RELATED TO EXPOSURE TO DISINFECTION BY-PRODUCTS

Cancer

The health effects of DBPs in drinking water have been a concern since DBPs were first reported in the 1970s. Early studies focused on cancer outcomes, while the more recent studies have focused on reproductive outcomes (IPCS 2000). According to the recent review by IPCS (2000):

more studies have considered bladder cancer than any other cancer. The authors of the most recently reported results for bladder cancer risks caution against a simple interpretation of the observed associations. The epidemiological evidence for an increased relative risk for bladder cancer is not consistent—different risks are reported for smokers and non-smokers, for men and women, and for low and high water consumption. Risk may differ among various geographic areas because the DBP mix may be different or because other water contaminants are also present. More comprehensive water quality data must be collected or simulated to improve exposure assessments for epidemiological studies.

The document also mentioned the difficulties in exposure assessment for epidemiological studies of cancer and DBPs, due to the long lag periods and the general lack of detailed historical data.

A very important recent pooled analysis by Villanueva *et al.* (2004), which provided quantitative information, confirmed this. For men there was an exposure response relationship between DBP intake and bladder cancer, but there was no relationship in women (Table 1). Furthermore, the latest Spanish study suggested that not only is exposure through ingestion an important risk factor but also exposure through swimming, showering and bathing (Villanueva *et al.* 2007). Furthermore in this study the authors identified genetically susceptible groups such as those with glutathione S-transferase theta 1 (GSTT1) and glutathione S-transferase zeta 1 (GSTZ1) polymorphisms (Cantor *et al.* 2006). Some studies have suggested an association between

Table 1 | Pooled analysis of bladder cancer and THM (after Villanueva *et al.* 2004)

THM exposure level (mg)	Male ORs (95%CI)	Female ORs (95%CI)
0–15	1.00	1.00
>15–50	1.22 (1.01–1.48)	0.92 (0.65–1.32)
>50–400	1.28 (1.08–1.51)	0.94 (0.70–1.27)
>400–1000	1.31 (1.09–1.58)	1.02 (0.74–1.41)
>1000	1.50 (1.22–1.85)	0.92 (0.65–1.30)

OR (95%CI) = odds ratio (95% confidence interval).

DBPs and colorectal cancers, while others have not (Young *et al.* 1981, 1987; Wilkins & Comstock 1981; Doyle *et al.* 1997; Koivusalo & Vartiainen 1997; Hildesheim *et al.* 1998; King *et al.* 2000a; Bove *et al.* 2007). Studies on colorectal cancer have relatively limited sample size and have used relatively crude measures of exposure assessment focusing principally on THMs levels in the water, without examining different exposures or gene–environment interactions. There is little evidence for an association between exposure to DBPs and other cancers such as liver, kidney, brain, lung and breast cancer, lymphomas or cancer of the pancreas, but the number of studies is small (IPCS 2000) and very few of these have involved populations in Europe. A recent report suggested an association between THMs and skin cancer, but further work needed to be conducted (Karagas *et al.* 2008).

Reproductive outcomes

Reproductive health outcomes should be easier to study from an exposure point of view, because of the shorter relevant exposure period. Among others, birth weight, prematurity, spontaneous abortion, congenital anomalies and stillbirth have been the focus of these studies. Overall there appears to be some evidence for a relationship between chlorination by-products, as measured by THMs, and small for gestational age (SGA)/intrauterine growth retardation (IUGR) and preterm delivery, but evidence for other outcomes such as low birth weight (LBW), stillbirth, congenital anomalies and semen quality appears to be inconclusive and inconsistent (Kramer *et al.* 1992; Aschengrau *et al.* 1993; Bove *et al.* 1995, 2002; Savitz *et al.* 1995, 2006; Kanitz *et al.* 1996; Reif *et al.* 1996; Gallagher *et al.* 1998; Waller *et al.* 1998, 2001; Dodds *et al.* 1999, 2004;

Klotz & Pyrch 1999; Magnus *et al.* 1999; King *et al.* 2000b, 2005; Kallen & Robert 2000; Yang *et al.* 2000, 2007; Nieuwenhuijsen *et al.* 2000b, 2008; IPCS 2000; Gevecker Graves *et al.* 2001; Jaakkola *et al.* 2001; Dodds & King 2001; Cedergren *et al.* 2002; Hwang *et al.* 2002; Fenster *et al.* 2003; Hwang & Jaakkola 2003; Shaw *et al.* 2003; Wright *et al.* 2003, 2004; Aggazzotti *et al.* 2004; Yang 2004; Hinckley *et al.* 2005; Porter *et al.* 2005; Toledano *et al.* 2005; Lewis *et al.* 2006, 2007; Tardiff *et al.* 2006; Luben *et al.* 2007).

Infante-Rivard (2004) found that the association between THMs and intrauterine growth retardation was modified by a metabolic polymorphism, with newborns without the CYP2E1 (G1259C) variant at high risk, but found no indication that *MTHFR* C677T modified the effect of exposure to chloroform and risk to foetal growth in humans. Neither did Shaw *et al.* (2003) for neural tube defects (NTDs). This sheds some light on the possible mechanism of action. However, the mechanisms through which DBPs may cause adverse health effects, including cancer and adverse reproductive effects are not well investigated. Several mechanisms have been suggested that involve genotoxicity (DeMarini *et al.* 1997; Pegram *et al.* 1997; Ross & Pegram 2004; Richardson *et al.* 2008), oxidative stress (Tomasi *et al.* 1985; Larson & Bull 1992; Ni *et al.* 1996; Scholl & Stein 2001; Meek *et al.* 2002; Gemma *et al.* 2003; Sciuto *et al.* 2003; Weber *et al.* 2003; Myatt & Cui 2004; Crider *et al.* 2005; Engel *et al.* 2005a,b; Min *et al.* 2006), disruption of folate metabolism (Kamen 1997; Ray & Laskin 1999; Dow & Green 2000; Geter *et al.* 2005), disruption of the synthesis and/or secretion of placental syncytiotrophoblast-derived chorionic gonadotropin (Chen *et al.* 2003, 2004) and lowering of testosterone levels (Potter *et al.* 1996).

Limitations

The major limiting factor in these studies has generally been crude exposure assessment. Use of ecologic water supply zone estimates as an exposure index may result in exposure misclassification. Furthermore, ingestion has generally been the primary interest, while uptake through showering, bathing and swimming is considerable (Whitaker *et al.* 2003). Combining information on individual water use with water zone estimates would provide more detailed exposure

assessment, if done appropriately, taking into account classical and Berkson error models (Nieuwenhuijsen *et al.* 2000b). Exposure estimates have been based primarily on residence. This ignores any exposure which occurs outside the home (e.g. in the workplace) and also ignores the possibility that a mother has moved house during her pregnancy. Exposure assessment based on residence therefore, results in exposure misclassification.

Most of the epidemiological studies have used THMs as a proxy for total DBP load, but THMs are not necessarily a good proxy measure. The metabolism of different DBP species varies (IPCC 2000), so it is insufficient to analyse DBPs as a whole, or to use TTHM (total THM) as a proxy. Investigation of the relation between non-THM by-products and reproductive outcomes is required in order to help elucidate the specific DBPs driving the associations observed.

In addition, when chlorine dioxide is used as disinfectant agent, chlorite and chlorate are the main DBPs; the toxicological action due to chlorite and chlorate has not yet been fully investigated. Only one study has been carried out in Europe on the association between personal exposure to these DBPs and pregnancy outcomes. This study was carried out in nine Italian provinces and evidenced a small increase in the risk of SGA at term (term SGA) and high levels of chlorite in drinking water (Aggazzotti *et al.* 2004).

Also, for reproductive epidemiological studies, in-depth analyses comparing exposure metrics for the different trimesters of pregnancy are required to discover the critical window in which DBP exposure affects foetal growth.

The retrospective and registry based nature of many of the reproductive epidemiological studies has meant that information on potential confounders, and other risk factors for foetal growth restriction, such as maternal smoking and alcohol consumption have often been lacking.

Furthermore, for reproductive epidemiological studies, there is also a need for better case identification for outcomes such as foetal growth restriction and congenital anomalies. Previous epidemiological studies have used a variety of outcomes as proxies for foetal growth restriction: term low birth weight (LBW), intrauterine growth retardation (IUGR) and small for gestational age (SGA). There are some limitations to these measures. LBW is rather

crudely defined; the fixed criterion of birth weight below 2,500 g takes no account of population-specific birth weight distributions (Wilcox 2001). Somewhat confusingly, the terms IUGR and SGA have been used interchangeably in the literature and criteria for IUGR/SGA diagnosis have varied, some studies using the 5th and some the 10th percentile of gestational specific weight according to a standard population growth chart as a cut-off point. These measures fail to distinguish between those babies which are constitutionally small and those which are pathologically small (i.e. growth restricted). Some small but normally grown babies will fall below the cut-off point, and some growth restricted babies will reach a weight above the cut-off point. A proportion of infants therefore are misclassified, and in epidemiological studies this may bias any association towards the null. There is evidence to show that the use of customised foetal growth charts, which take into account factors such as maternal height and ethnicity, significantly reduces the proportion of false-positive and false-negative diagnoses of foetal growth restriction, compared with the use of a standard population growth chart (Gelbaya & Nardo 2005; Gardosi 2006).

Congenital malformations have often been classed into main categories (e.g. neural tube, major heart and abdominal defects) as a result of the small number of cases in the studies. These malformations, however, are generally heterogeneous with respect to both phenotype and presumed aetiology. Nieuwenhuijsen *et al.* (2008) showed that focusing on isolated subcategories may result in different findings.

Even though there has been some good animal work that suggests strong effects on semen quality (Smith *et al.* 1989; Toth *et al.* 1992; Linder *et al.* 1994a,b, 1995, 1997a,b), only two small US epidemiological studies have been conducted (Fenster *et al.* 2003; Luben *et al.* 2007).

The pooled bladder cancer analysis results have provided good evidence for some risk related to DBPs; however, the authors did not examine whether the results in North America and Europe were consistent (Villanueva *et al.* 2004). There may be differences because of different water treatment practices. Further, the results for colon cancer have been inconsistent and inconclusive and have not examined the role of different exposure pathways and routes, which may be important.

Very few studies have examined gene–environment interaction and/or the presence of susceptible groups (Shaw *et al.* 2003; Infante-Rivard 2004), which is important for guideline setting and can elucidate potential mechanisms.

THE HIWATE PROJECT

HIWATE is a major research initiative that has started in Europe (www.hiwate.org) to address the shortcomings of previous research on DBPs. The overall aim is to investigate potential human health risks (e.g. cancer, premature births, SGA, semen quality, stillbirth, congenital anomalies) associated with long-term exposure to low levels of disinfectants (such as chlorine) and DBPs occurring in water for human consumption and use in the food industry. The study will comprise risk–benefit analyses including quantitative assessments of risk associated with microbial contamination of drinking water versus chemical risk and will compare alternative treatment options. The outcome will be improved risk assessment/management. The study will make use of existing studies/databases and newly collected information. The project involves 16 teams in eight European countries (Table 2). The work is divided into different topics (exposure assessment, epidemiology, risk assessment and management) and studies (Table 3).

The specific objectives of the proposed work are outlined below.

Exposure assessment

(I) To determine the DBP composition and levels in drinking water in various regions in Europe (see Table 4).

Representative water samples will be primarily collected in the regions where the epidemiological studies are carried out, to give a wider picture on their presence and levels. Samples will be analysed for THMs (chloroform, bromodichloromethane, chlorodibromomethane and bromoform), haloacetonitriles (HANs) (including chloroacetonitrile, dichloroacetonitrile, trichloroacetonitrile, bromoacetonitrile, dibromoacetonitrile, tribromoacetonitrile, chlorobromoacetonitrile, dichlorobromoacetonitrile and dibromochloroacetonitrile), HAAs (including chloroacetic acid, dichloroacetic acid, trichloroacetic acid, dibromoacetic acid, tribromoacetic

Table 2 | Participants in HIWATE

Participant organisation name	Short name organisation	Country
Centre for Environmental Epidemiology	CREAL	Spain
Imperial College London	Imperial	United Kingdom
University of the Aegean	UA	Greece
National Public Health Institute	KTL	Finland
Vytautas Magnus University	VMU	Lithuania
University of Crete	UC	Greece
Université de Rennes	INSERM	France
Municipal Institute of Medical Research Foundation	FIMIM	Spain
Centre for Genomic Regulation	CRG	Spain
University of Modena and Reggio	UMR	Italy
Istituto di Ricerche Farmacologiche 'Mario Negri'	MN	Italy
Swedish Institute for Infectious Disease Control	SIIDC	Sweden
Hylobates Consulting Srl	HCS	Italy
ICON Ltd	ICON	United Kingdom
Scarab	Scarab	Sweden
Catalan Institute of Cancer	ICO	Spain

acid, dibromochloroacetic acid, chlorobromoacetic acid, bromoacetic acid, dichlorobromoacetic acid), halo ketones (HAKs) (including 1,1-dichloropropanone, 1,3-dichloropropanone, 1,1,1-trichloropropanone), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), chlorate hydrate (CH), chloropicrin (CP), bromate, chlorite and chlorate, depending on the type of water disinfectant treatment used. The study will produce a database containing the levels of these DBPs in the various regions in the UK, France, Spain, Greece, Italy and Lithuania. The number of samples collected in each region is given in Table 4. The samples will be collected over a two year sampling period to provide information on the temporal variability of the DBPs over different seasons. Some work will be done to examine the effects of filters and boiling water. Detailed sampling and analyses protocols have been developed (see www.hiwate.org). Furthermore, an interlaboratory comparison programme will be set up to compare the performance of the various laboratories within the HIWATE consortium with laboratories outside the HIWATE consortium.

(II) To identify the determinants of DBPs and develop predictive models.

In addition to the DBP analysis for a range of DBPs (see objective 1), the study will obtain information regarding the

possible determinants of the DBPs including organic matter content, water source, temperature, pH and (residual) disinfectant levels (e.g. chlorine and bromide level) (Table 5). Statistical techniques will be employed to quantify the effect of these determinants on the formation of DBPs and use this to build a predictive model of DBP formation (Golfinopoulos & Arhonditsis 2002; Nieuwenhuijsen 2003; Nikolaou *et al.* 2004; Whitaker *et al.* 2005). Furthermore, the correlation between THMs and other DBPs will be assessed.

Initially a separate hierarchical model will be built to describe the data originating from each of the regions/countries. These region-specific models will be of a similar structure but the determinants included in the final models of each region may differ. We will explore ways in which these region-specific models might be combined, for instance by including an extra level in the hierarchy of the model structure. This extra level will allow us to explore the variability between the regions.

Basic mixture model

For each DBP and each region we will build a separate hierarchical model in order to describe the temporal and

Table 3 | Studies by topic area in the HIWATE study

Exposure assessment studies	Epidemiological studies	Health risk assessment and management
DBP measurements in UK, France, Spain, Greece, Italy and Lithuania (WP1)	A nation-wide study of congenital anomalies in the UK (approx 20,000 cases and 2.5 million births) (WP3)	A risk-benefit analysis study including quantitative assessments of risk associated with microbial contamination of drinking water versus chemical risk, compare alternative treatment options, and produce burden of disease estimates in Barcelona, Bradford, Rennes, Heraklion, Kaunas and Modena (WP8)
Modelling of various DBPs using data from WP1 and information determinants of the DBPs (WP2)	A study of congenital anomalies in the Emilia Romagna region in Italy (approx 150,000 births) (WP3)	
	A water treatment intervention study of stillbirth and low birth weight in the UK (approx 360,000 births) (WP3)	
	A study of small for gestational age and premature birth in five pregnancy cohorts in the UK, Spain, Greece, France and Lithuania (23,000 births) (WP4)	A review of the water and health policies in Europe, USA and worldwide in relation to water disinfection (WP9)
	A case-control study of semen quality in the UK (1,700 cases and controls) (WP5)	A guide to best practice in terms of water disinfection and a brief assessment of disinfection alternatives (WP9)
	A pooled analysis of European bladder cancer studies with almost 6,000 cases and controls (WP6)	Organise a workshop to bring together scientists working on environmental, toxicological, epidemiological and policy aspects of chlorination DBPs, microbiologists, policy-makers, and representatives from the water industry and consumer organisations in Europe to develop guidelines for policy across Europe and the future research agenda (WP9)
	A case-control study of colon cancer in Italy and Spain (2,000 cases and controls) (WP7)	

Table 4 | Proposed number of samples to be collected in various regions in Europe

For which workpackage/study	Number of DBP samples analysed by University of Aegean, Greece	Number of samples MX analysed by KTL, Finland (first year)	Number of bromate samples analysed by KTL, Finland	Chlorite/chlorate, analysed by University of Modena and Reggio, Italy
WP3 congenital anomalies, Italy	20 (10 Emilia Romagna, 5 Milan, 5 Friuli)	20 (7) (10 Emilia Romagna, 5 Milan, 5 Friuli)	20 (10 Emilia Romagna, 5 Milan, 5 Friuli)	100 chlorate Emilia Romagna, 50 Milan, 50 Friuli
WP3 + WP5 congenital anomalies, low birth weight, stillbirth and semen quality, United Kingdom	100	10 (3)	4	
WP4 Birth weight and prematurity, Bradford, United Kingdom	150	10 (1)		
WP4 Birth weight and prematurity, INMA study areas, Spain	200	10 (3)	4	Some chlorite/chlorate
WP4 Birth weight and prematurity, Pelagie study area, Rennes, France	60 smaller network 40–80 bigger network	10 (3)		
WP4 Birth weight and prematurity, RHEA study, Crete, Greece, Evripidis, stephanou@chemistry.uoc.gr	150	3 (3)		
WP4 Birth weight and prematurity, Kaunas, Lithuania	144	8 (4)	4	
WP6 bladder cancer areas	No additional (to be re-evaluated for specific areas Spain)			
WP7 Colon cancer, Barcelona, Spain	114	10 (3)		
WP1 other, e.g. Athens	48			
WP1 boiling/filter experiment	To be defined			
Total	1,066	81 (27)	32	

Table 5 | Sampling form

<i>WORK PACKAGE 1 (WP 1) DETERMINATION OF DBPs COMPOSITION AND LEVELS IN VARIOUS REGIONS IN EUROPE</i>				
COUNTRY				WP
Sample Ref. ID				
Date and time	Sampling	Shipping	Arrival	Analysis
Company				Sampling site
Water source	Surface	Groundwater	Lowland	Highland Mixed
Treatment processes (pre-treatment/coagulation/flocculation-sedimentation/filtration/activated carbon)				
Disinfection process (chlorine/chloramines/chlorine dioxide/ozone/UV)				
Distribution system	Length (m)	Type	Ring	Tree
Treatment plant capacity (m ³ /day)				
Population served				
Location (address, post code, water supply zone)				
Reaction time (h)				
Residence time (h)	Flow (m ³ /h)	Distance (m)	Pipe Dimension (m ³)	
Temperature (°C)	Raw		Tap	
pH	Raw		Tap	
Chlorine dose (mg/l)	Prechlorination		Postchlorination	Network chlorination
Free residual chlorine (mg/l)				
Coagulant dose (mg/l)				
Bromides (mg/l)	Raw		Tap	
TOC (mg/l)	Raw		Tap	
UV absorbance (mg/l)	Raw		Tap	
DBPs (µg/l) (Measured from water treatment plant)				

spatial variability of the data in that region. We will follow the basic hierarchical structure as used by Whitaker *et al.* (2005) in modelling THMs. The data will be transformed so that the values of each DBP that we model are approximately normally distributed.

In previous work modelling THMs it has been found that a mixture model is necessary. This form of model is particularly suited to non-normal distributions where the underlying data may have arisen from a number of distinct sources or populations. In Whitaker *et al.* 2005, the model assigns a water source type, or some mixture of types (for instance ground, lowland surface or upland surface), to each water supply zone. For each DBP model we will explore the different number of components needed in the mixture model. It may be desirable to use jump Markov Chain Monte Carlo techniques to allow the number of components to be estimated in the model. For some DBPs a mixture model may not be required at all. Seasonal variation is then taken into account by adding a quarterly effect common to all zones supplied by the same source type.

Furthermore, measurements under the detection limit will be modelled to obtain an estimate between 0 and the detection limit, rather than arbitrarily assigning a value of, for example, half the detection limit. In this approach a zone mean depends on measurements taken within that particular zone and the DBP levels for water of the same source type in other zones, taking into account seasonal variability across the region. This model can then be used, after it is back-transformed onto the original scale, to predict quarterly zone specific estimates of the DBP of interest. The model produces robust estimates for each zone together with an estimate of the degree of uncertainty around the estimates.

One of the main advantages of the model is that it provides good estimates for zones where few or no measurements are available. This hierarchical modelling approach fits well into a Bayesian framework and the software WinBUGS (Bayesian analysis using Gibbs sampling) will be used for the estimation (Spiegelhalter 2003). These techniques have already been successfully applied elsewhere and provide a cost efficient way to provide exposure estimates for current and past exposures for epidemiological and risk assessment studies in areas where information on potential determinants is available, but where

there is no or little information on DBP levels. In order to visualise the modelled DBP estimates and check for any unusual estimates or potential errors in the modelling, the modelled quarterly zone mean DBP estimates will be classified into exposure categories. The categorised DBP estimate for each zone and quarter will then be mapped for each water region, together with the raw annual mean DBP. These will then be sent to the local water utilities for checking.

Regression modelling

The basic model described above aims to explain geographical and seasonal variability. This model will then be extended by incorporating factors affecting the creation of the relevant DBP. There is a wide literature on the determinants of DBP concentrations in water (see Sadiq & Rodriguez 2004 for an overview).

By adding additional regression terms to the model described above we will seek to further explain the variability in the relevant modelled DBP. In determining the form of these regression models we will follow the regression mapping methodology used in this type of study (Sadiq & Rodriguez 2004). Here, the fitted DBP levels at each sampling point, as produced in the above hierarchical model, are treated as fixed and become the dependent variable in a regression analysis against possible DBP formation determinants.

A simple statistical regression model can be expressed in the form:

$$\ln(C_{ij}) = \beta_0 + \beta_1 \text{var}_i + \beta_2 \text{var}_j + E$$

where $\ln(C_{ij})$ denotes the log transformed exposure concentration, β_0 the background level, var_x the potential determinant of exposure, β_x the regression coefficient of var_x providing the magnitude of the effect, and E a random variable with mean 0, often called the error term.

Further, levels can be added to take account of multi-level effects.

Epidemiology

(III) To assess the risk of reproductive effects in relation to disinfection practices and levels of disinfection by-products,

epidemiological studies will be conducted to examine the relationship between DBP exposure estimates and a number of outcomes.

(a) Congenital anomalies, including neural tube, major heart, major stomach wall, and urinary tract defects, cleft palate/lip will be studied in a large, nation-wide, cross-sectional study, using registry data in the UK, where mainly chlorination is used as a disinfectant. The study included over 2.5 million births and approximately 20,000 cases with congenital anomalies. The study uses novel Bayesian statistics for the exposure assessment modelling (Whitaker *et al.* 2005). Initial results have been published and showed no association between THMs and cleft palate/lip, abdominal wall, major cardiac, neural tube, urinary and respiratory defects, except for a restricted set of anomalies with isolated defects. There were excess risks in the highest exposure categories of total THMs for ventricular septal defects, OR (odds ratio) = 1.43 (95% confidence interval (CI) 1.00–2.04) and of bromoform for major cardiovascular defects and gastroschisis, OR = 1.18 (95% CI 1.00–1.39) and OR = 1.38 (95% CI 1.00–1.92), respectively (Nieuwenhuijsen *et al.* 2008).

Congenital anomalies, including neural tube, major heart and urinary tract defects will also be studied using registry data in Italy in the Emilia Romagna region, where mainly chlorine dioxide is used as a disinfectant. The study includes around 150,000 births and will be analysed as a case-control study (1:2 ratio). The main exposure variables are the concentrations of some DBPs (THMs, chlorite and bromate) in drinking water networks supplying the homes of the mothers of cases and controls during the first trimester of pregnancy. Moreover, other information on determinants of DBPs will be collected. On the basis of each subject's home address the local water network supplying drinking water during the period of interest will be identified. The following data on waterworks will be collected: type of water source, disinfection treatment and supplied population. The following analytical data will be collected: total and individual THMs (chloroform, bromoform, bromodichloromethane (BDCM), chlorodibromomethane (CDBM)), chlorite, bromate, nitrate, residual disinfectant, total organic carbon, oxidant power, pH and hardness. Information on potential confounders will be collected, such as: social and demographic variables of

mother and father (residence and address, age in years, nationality, education, occupation, blood relationship); reproductive history of the mother (parity, number of previous live births, stillbirths, previous terminations); present pregnancy (date of last menstrual period, obstetric history, hospital admissions); termination (date...); delivery (date, single/plural births, live birth, stillbirth, gender, weight, length, cranial circumference); and stillbirth (causes according to international classification of diseases (ICD) 9).

(b/c) Stillbirth and LBW will be studied in an intervention study in the North East of England in the UK, in areas where enhanced coagulation in the water treatment plant was installed in 2003. The rates of stillbirth and low birth weight will be examined 3 years before and after the intervention. In each year there are around 60,000 births. Primary analysis will aim to determine whether there is evidence for a reduction in the rates of stillbirth after introduction of the new water treatment practices compared with before the changes took place. Secondary analysis will focus on specific THM species. Differences in small-area rates of stillbirth before and after treatment changes will be modelled against change in total and individual mean annual THM concentrations using Poisson regression. For each $\mu\text{g l}^{-1}$ decrease in THM concentration, the increase/decrease in rates will be determined. In addition, areas will be categorised into low, medium and high change in THM concentrations and the change in rates of stillbirth will be estimated in each category using Poisson regression. Models will be adjusted for potential confounders such as maternal age and social deprivation (Carstairs' deprivation score and/or index of multiple deprivation).

(d) SGA, FGR and premature birth will be studied in five pregnancy cohorts in the UK, Spain, Greece, France and Lithuania (Table 6), where a range of treatments are used.

Cohort in France (Pelagie). The ongoing French epidemiological PELAGIE study (*Perturbateurs endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance*) is a follow up study conducted from 2002 onwards in three departments of Brittany (France): Ille-et-Vilaine, Côtes d'Armor and Finistère. Recruitment of study subjects finished at the end of 2005, with a population

Table 6 | Pregnancy cohorts included in the HIWATE study

Cohort	Total subjects with questionnaire data	Subjects with questionnaire, and cord and mother's blood	Subjects with questionnaire and only cord blood	Subjects with questionnaire and only mother's blood
France, Pelagie	3,500		1,500	
Greece, Rhea	1,700	1,700		
Lithuania, Kaunas	4,000			4,000
Spain, INMA	2,500	2,500		
UK, BiB	10,000	10,000		
Total	23,000	15,500	1,500	4,000

of 3,500 study subjects (80% participation rate). The general objective of the study is the assessment of exposure during pregnancy to environmental and occupational pollutants, and evaluate the association with reproductive adverse effects such as intrauterine growth retardation/small for gestational age, low birth weight, prematurity and congenital malformations. Mothers are recruited in the first trimester of pregnancy through a gynaecologist/obstetrician, general practitioner or echographer. Then, they are administered a questionnaire and urine samples are collected. At birth, samples of placenta, mother hair and cord blood are obtained. Six months after birth, a neurological test is administered to the child. The existing exposure assessment: 1) compares regulatory with ad hoc measurements; 2) evaluates seasonal and geographical variability of THM levels; and 3) evaluates the relevance of personal habits (drinking water, showering, bathing, etc.) in the assessment of THM exposure. In the study area, 150 tap water samples were collected: 100 during October–November 2004 and 50 during April–May 2005. Individual questionnaires to collect data on, for example, water consumption, frequency and duration of showers, baths and swimming pool attendance have been distributed.

Cohort in Spain (INMA study). The Spanish birth cohort, called INMA–*Infancia y Medio Ambiente* (Environment and Childhood), is a network of research groups in Spain that have built up a project aiming to study the role of the most important environmental pollutants in air, water and diet, life-style and socioeconomic conditions during pregnancy and early in life and their effects on child growth and development (Ramon *et al.* 2005). It is a prospective population-based cohort study. Pregnant women are

assessed at 12, 20 and 32 weeks of gestation to collect information about environmental exposures and foetal growth, and to obtain maternal venous blood samples (20 ml). For the DBP analysis, the INMA project will follow up a population sample of 2,500 pregnant mothers and newborns recruited in four study areas: Basque country (N = 500, enrolment 2005–2007), Valencia (N = 800, 2003–2005), Asturias (N = 500, 2005–2007) and Sabadell/Barcelona (N = 800, 2005–2007) (participation rate approximately 80%). The main exposures of interest in the study are environmental contaminants in air and water (trihalomethanes), persistent and semi-persistent pollutants in different biological samples, maternal occupation, diet and dietary determinants such as antioxidants, folate and fatty acids, genetic determinants, social determinants including parental education, marital status, employment status and paternal-to-child attachment and paternal mental status. For the exposure assessment to disinfection by-products, tap water samples will be taken from the study areas to measure trihalomethanes. They will also collect available data from water companies and local authorities. The study population are administered a questionnaire including data on water consumption and water-related habits (e.g. showering, bathing, swimming pool use).

Cohort in Greece (Rhea study). The Greek birth cohort was initiated on the island of Crete and will enrol all births in one year within the prefecture of Heraklion, which includes urban and rural areas with different water supply sources (N = 1,700). About one-third of the subjects live in rural or semi-urban areas and about one-fifth of the pregnant mother are recent immigrants. The existence of a well-developed health care system in Crete provides an

advantage for the identification and close follow-up of a cohort in a relatively closed population. On the basis of pilot studies, an 80% participation rate is expected for both questionnaire and biological sample collection. The majority of study subjects will be identified through four main hospitals in Heraklion. Information will be collected on life-style factors, occupational and environmental exposures and nutrition, which are predominantly based on a Mediterranean diet. Follow-up will combine computerised archives with active contacts following procedures applied in previous children cohorts in Crete. Sources of water differ substantially in the areas of the study and will provide a population with contrasted exposures. Analyses of DBP levels in the past have indicated the presence of DBPs at levels below those in other Mediterranean coastal areas while relatively high levels of brominated compounds have been identified. Subjects will be personally interviewed with a computerised interview regarding sources of water, and other habits related to use of water such as showers, swimming pools or contact at work. Biological samples will include blood samples from the mother, cord blood, and child at age four, urine samples from the mother at pregnancy, hair of the child, and toenail (mother).

Cohort in Lithuania. Kaunas is a second city of Lithuania with 400,000 inhabitants and 4,000 births per year. The Lithuanian epidemiological study is a population-based cohort study that includes all pregnant Kaunas city women in 2007–2008 ($n = 4,000$). The main objective of the study in Kaunas is to identify the environmental factors that are associated with newborns' development and early childhood allergy. Pregnant women will be recruited through antenatal clinics in the city. Mothers will be identified in the first trimester of pregnancy, mainly through a general practitioner or gynaecologist and will be interviewed. The health institutions in Kaunas that register mothers include four clinical hospitals, 19 outpatient departments, nine private treatment centres and 15 family health centres. A second interview will take place in the four main Kaunas hospitals' maternity departments. Exposure assessment for the critical trimester of pregnancy will be based on personal information on water consumption and other THMs-related activities obtained through questionnaire, and water work-level information on water quality—both routinely collected

information and based on water quality analyses of THMs and exposure modelling. There are four water utility networks that supply underground water treated by sodium hypochlorite. Blood samples of the mother will be collected for genotyping. Information on potential confounders and modifiers (health behaviour, job exposures, sociodemographic data) will be collected prospectively, during interview by standardised questionnaire.

Cohort in the UK (Born in Bradford). The study population is to be drawn from the metropolitan district of Bradford in the UK. Bradford is the eighth most deprived health community in the UK with an infant mortality rate which is significantly higher than the UK average. A greater proportion of babies born in Bradford are of low birth weight (9.7%) compared with England and Wales as a whole (7.5%). Nearly 50% of the 5,500 babies born each year in Bradford are to parents of South Asian origin. The high prevalence of low birth weight and ethnicity in the Bradford community provides a unique setting in which to investigate causes of foetal growth restriction and low birth weight. Study families (mother, father and index child) will be recruited by the Born in Bradford (BiB) prospective cohort study. The study aims to investigate risk factors for abnormal foetal growth and birth outcomes. Recruitment is began in February 2007, and it is aimed to recruit 10,000 families over a 2-year period. Participants will be enrolled at the antenatal glucose tolerance test (26–28 weeks gestation).

Pooled analyses. We expect to be able to extract from the existing cohort studies (France, Spain) and obtain from the new cohort studies (Lithuania, Greece, UK) around 23,000 births for pooled analysis (Table 6). All subjects will have complete questionnaire data and in most cases both cord blood and mother's blood will be available.

Exposure assessment. The five studies include questionnaires that cover several different areas such as socio-demographic, life-style, nutrition, occupation, medical and reproductive history, family history, environmental exposures and other. The questionnaires used in the studies in Crete and Spain are fairly similar. All studies have information on water intake and sources of drinking water.

In addition, all studies have information on showers, baths and swimming pool use during pregnancy. The degree of detail, however, of this information differs considerably between studies, and an effort will have to be made to adapt some of the questionnaires. One set of analyses will be based on the average level of THMs during pregnancy based on routinely collected THMs for regulatory purposes, and indices based on the combination of THM measurements and personal activities such as ingestion, showering, bathing and swimming as an estimate of total dose. This analysis will be completed with information from measured DBPs under Work Package 1 that will include several other compounds apart from THMs. This information will be modelled (WP2) based on available water quality parameters, treatment and water source for the study regions. Exposure categories will be formed (e.g. none, low, medium and high) for initial analysis, followed by continuous indices, if appropriate. The cohorts in Crete, Bradford and Kaunas will measure many DBPs (through WP1) during the subjects' pregnancy, while INMA and PELAGIE have measurements on THMs and will collect information on many DBPs (through WP1) after the subjects' pregnancy. Modelling techniques will be used (through WP2) to obtain estimates on various DBPs for all the subjects during the whole length of pregnancy.

Various exposure indices will be used including average exposure over the whole pregnancy and also average exposure during the first, second and third trimesters. Use of trimester-specific exposure estimates will allow evaluation of the critical exposure window. The questionnaires of all cohorts include information on the main confounders of interest such as maternal age and education, socioeconomic status, parity, smoking and alcohol consumption.

The outcomes that will be measured are:

- low birth weight (LBW)
- small for gestational age (SGA) including symmetrical and asymmetrical SGA
- preterm delivery
- foetal growth restriction (FGR) → preferential measure
- parameters derived from the ultrasounds

In addition to DBP metabolising genes, a series of other genes will be selected that may influence reproductive outcomes through other mechanisms such as genes on

oxidative stress and related to the folate-mechanism (e.g. MTHFR). To interpret the function of some of these genes information should be available through the nutritional questionnaire on folate and multivitamin use during the pregnancy since that may have a modifying effect. Candidate genes will be identified on the basis of their reported involvement in the metabolism of DBPs (i.e. their potential interaction with environmental exposures). The criteria used for the selection of candidate genes will be based on reported biological and genetic relevance (e.g. <http://www.cdc.gov/genomics>): (i) evidence from epidemiologic studies on disease association and gene–environment interaction; and (ii) evidence of the involvement of the genes in any reproductive outcome pathobiological pathway. Selection of specific SNPs (single nucleotide polymorphisms) in the genes or regions of interest will be based on established criteria, including ethnicity, population frequency (e.g. MAF—minor allele frequency—above 10% for most SNPs), validation status, location and type of sequence (e.g. coding sequences, promoter regions, 5'UTR and 3'UTR, splicing regions, etc.) and reported or predicted function (e.g. SNPs evolutionary conserved, SNPs in well-defined domains, etc.). The final selection of genes and SNPs to be analysed will be decided at a later stage. The genes to be analysed will include CYPE1, GSTT1, GSTZ and others.

(e) Semen quality will be studied using an existing case-control study (CHAPS-UK) (Clyma *et al.* 2008) in the UK, where mainly chlorination is used for water disinfection. Subjects were drawn from new patients attending fertility clinics for investigation: sperm donors were specifically excluded. Cases were new male patients seen at any of the clinics over a 25 month period who had $<12 \times 10^6 \text{ ml}^{-1}$ progressively motile sperm in their initial semen sample. Around 1,700 cases and controls have been recruited. The study uses novel Bayesian statistics for the exposure assessment modelling (Whitaker *et al.* 2005) and the exposure and health data will be linked in GIS (geographic information system). Information on potential confounders has been collected and the analyses of semen quality and DBP levels will be adjusted for potential confounders in logistic regression models. Unfortunately no information is available on the various exposure pathways and routes and only DBP concentrations in the water will be used as an exposure index for the critical exposure windows.

(IV) To assess the risk of cancer, particularly bladder cancer and colon cancer, in relation to disinfection by-product practices and DBP levels, including the examination of any gene–environment interactions (e.g. CYP2E1, GSTT1).

The study will obtain risk estimates from existing case-control bladder cancer studies in Spain, France (includes ozonation as treatment) and Finland, and produce specific risk estimates for Europe. The work will build on a pooled analysis that has been conducted examining long-term exposure to chlorination by-products by combining residential information from questionnaires with information from water utilities gathered in six case-control studies from the US, Canada and Europe (Villanueva *et al.* 2004). It included 2,806 cases of bladder cancer and 5,254 controls. The Finnish case-control study contained 732 bladder cancer cases and 914 controls (Koivusalo *et al.* 1998). The French study was a hospital-based case-control study of bladder cancer conducted between 1985 and 1987, including 765 cases and 765 controls (Chevrier *et al.* 2004). The Spanish study is the most recent and included 1,226 cases and 1,271 controls (Villanueva *et al.* 2007). The cases and controls have been genotyped (e.g. CYP2E1, NAT2, GSTM1 and GSTT1), funded by the National Cancer Institute, and the results will be included in the current study (Garcia-Closas *et al.* 2005). The current study will compare and contrast risk estimates from the above studies and the recently conducted pooled analysis to obtain the best or a range of risk estimates for various disinfectant practices and DBPs for the risk–benefit analysis, including genetically susceptible populations.

A case-control study will be conducted to examine the relationship between DBPs and colorectal cancer in Spain and Italy. The main aims are the evaluation of the long-term exposure to various DBPs in the study subjects through ingestion, inhalation and dermal absorption and the risk of colorectal and rectal cancers associated, including the examination of any gene–environment interactions. There will be 500 cases and 500 controls in Italy (areas of great Milan and the provinces of Pordenone and Udine) and in Spain (Barcelonès, Baix Llobregat, Vallès Occidental, Maresme, and Vallès Oriental, in Barcelona province), bringing the total study population to 1,000 cases and 1,000 controls. Study subjects will be interviewed

face-to-face using a structured questionnaire administered by trained interviewers. The questionnaire includes information on socio-demographics, smoking habit, coffee and alcohol consumption, diet, physical activity, occupational exposures, medical history and drug use, family history of cancer, and detailed information on water use and water-related habits: drinking water source at each residence from birth (municipal/private well/other); quantity and type (bottled/tap) of water consumed, including water based fluids (coffee, tea and herbal infusions); average frequency and duration of showering and bathing; lifetime swimming pool attendance; and dish washing habits. Main potential confounders and covariates are included in both questionnaires. Each centre has included questions on other potential risk factors that are not the main focus of this proposal (e.g. drugs, medical history, etc.). A blood sample will be collected from each subject. Retrospective information on water source, treatment and quality in the study municipalities will be obtained through a questionnaire aimed at water companies and local authorities. Tap water samples will be collected in the study areas to measure a range of DBPs (as part of WP1). Retrospective DBP levels in the study areas will be modelled on the basis of historical data on water source and treatment (see Villanueva *et al.* 2006). Data on DBP levels will be combined with personal information on water-related habits. Personal indices of exposure to DBP through different routes (ingestion, inhalation and dermal exposure) will be calculated. An overall index combining different exposure routes will be also calculated applying weighting factors obtained from the literature (see Villanueva *et al.* 2006).

Polymorphisms analysed include several types of marker: SNPs (single nucleotide polymorphism), In/Del polymorphisms (polymorphisms of short deletions or insertions) and large deletions (e.g. null GST alleles). The selected study design is the ‘candidate gene approach’ based on the analysis of those genes potentially involved in a functional way; for the first phase of the study we will focus on those involved in the DBP metabolism (e.g. GSTT1, CYP2E1, GSTZ1) and in folate metabolism (e.g. MFTHR). A comprehensive review will be conducted to identify key genes that may be involved in the interplay between DBP exposure and colorectal cancer risk. Candidate genes will be selected after discussion between partners.

Risk assessment and management

(V) To conduct risk–benefit assessment including quantitative assessments of risk associated with microbial contamination of drinking water versus chemical risk, compare alternative treatment options, and produce burden of disease estimates (e.g. DALYs, disability-adjusted life years).

The study will build on and make use of expertise and experience of EC-funded projects such as MICRORISK (www.microrisk.com) and INTARESE (www.intarese.org). The purpose of the assessment is defined as the following research question: ‘What is the net human health impact of microbial and disinfectant by-product contamination of drinking water?’ The *pyrkilo* methodology, an open risk assessment method, will be used to create an integrated risk–benefit model (Tuomisto & Pohjola 2007).

We will develop an overall framework for the risk–benefit analyses of microbial and chemical risks, specifically for DBPs in drinking water. The framework will integrate long-term chemical effects versus the short-term microbial effects to make realistic comparisons. We will conduct risk–benefit analyses, including quantitative assessments of risk associated with microbial contamination of drinking water versus chemical risk, compare treatment options (e.g. chlorination, chlorine dioxide and ozonation), and produce burden of disease estimates. The risk–benefit analyses will be the result of integrated DPBs and microbial risk assessments, from modelling of alternative treatment options and from different risk–benefit metrics, including burden of disease (e.g. DALYs). As far as we are aware only one such study has been reported in the literature, describing a risk–benefit model for *Cryptosporidium parvum* and bromate exposure and comparing the risks and benefits of ozonation using disability-adjusted life years (Havelaar *et al.* 2000).

The work will start with a review to identify the relevant microbial and DBP exposures and related diseases (e.g. infectious diarrhoea, gastrointestinal illness and reproductive and cancer outcomes, respectively). This will be followed by an exact framing of the risk assessment (more details in Meriläinen *et al.* 2008). All DBPs from the exposure assessment part of the HIWATE study (WP1 and 2) will be considered. Information on personal habits including, for example, ingested amounts of water,

showing, use of filters and boiling water will be obtained from the epidemiological studies and the EC-funded projects MICRORISK and INTARESE, for which this information was also collected, and from other available studies (Barbone *et al.* 2002; Kaur *et al.* 2004; Westrell *et al.* 2006) and will be organised in a meaningful and coherent framework.

DBP exposure and risk estimates, including exposure–response relationships of DBPs will be provided by the exposure assessment (WP1 and 2) and epidemiological research areas (WP3, WP4, WP5, WP6, and WP7) of the current proposal and from the literature, particularly where pooled or meta-analyses are available (e.g. for bladder cancer, Villanueva *et al.* 2004), or we have to rely on toxicological data. The outcome data for the risk–benefit analyses will be prioritised using set criteria. For outcomes such as cancer, long-term exposure will be taken into account. Where necessary, novel dose–response relationships for DBPs will be derived combining data from the epidemiological studies, from published toxicological and other relevant studies (see e.g. Peters *et al.* 2005).

Exposure estimates for microbiological load will come from routinely collected data (heterotrophic plate counts and indicator bacteria including coliforms, *Escherichia coli* and *Clostridium perfringens* as set out in Council Directive 98/83/EC) provided by water companies in the area, literature, or newly collected data, where necessary. The data will be linked with failure frequency distributions by converting indicator values into hypothetical input incidences in the distribution network (see e.g. Westrell *et al.* 2003). We will take into account the relationship with the raw water quality and its potential content and variability of microbial load (see e.g. Westrell *et al.* 2004). Dose–response relationships will come from MICRORISK and the literature.

The dose–response of and the barrier efficiency of other treatment steps for several other specific microbes causing waterborne diseases worldwide but not routinely measured (including Campylobacteraceae, Mycobacteria, *Giardia*, *Cryptosporidium*, protozoa and enteric viruses) for disinfection methods will be assessed (see e.g. Persson *et al.* 2005). Also, the indicator value of heterotrophic plate counts for pathogenic waterborne bacteria will be evaluated.

Risk estimates for related infectious diseases will come from the EC-funded MICRORISK, other sources or will be derived where not available. For the risk assessment, a combined or best dose–response will be selected based on the ability to predict cases in an independent data set (validation with one of the studies, see above). Before entering the risk–benefit (or risk–risk) analysis, areas of non-independence of the microbiological and chemical risks will be examined (e.g. same susceptible or highly exposed populations, correlation between high DBPs exposure and higher microbial load). In all the above work, variability and uncertainty will be incorporated in the models and sensitivity analyses will be conducted on the results.

As part of the study, the water consumption, water treatment techniques, treatment performance in water works and raw water quality will be evaluated in the case study areas: Barcelona, Bradford, Rennes, Heraklion, Kaunas and Modena. A few scenarios will be constructed for intervention, and the difference in the outcome measures estimated: a) change in treatment by water company; b) changes in

behaviour (e.g. change from tap to bottled water); and c) use of point-of-use measures (e.g. filters) where they are needed (specifically for food industry).

(VI) To review the water and health policies in Europe, USA and worldwide in relation to water disinfection.

Best practice in terms of water disinfection and a brief assessment of disinfection alternatives will complete the study. A final workshop will be organised in 2010 as an open conference that will aim to bring together scientists working on environmental, toxicological, epidemiological and policy aspects of chlorination DBPs, microbiologists, policy-makers, and representatives from the water industry and consumer organisations in Europe to provide information for the development of guidelines for policy across Europe and the future research agenda. Specific objectives include: comparison of policies related to DBPs in drinking water in Europe, North America and worldwide; review the current literature on toxicological and epidemiological findings of DBPs and adverse health outcomes, including findings from the HIWATE epidemiological studies; assessment of the findings of the HIWATE study in terms of

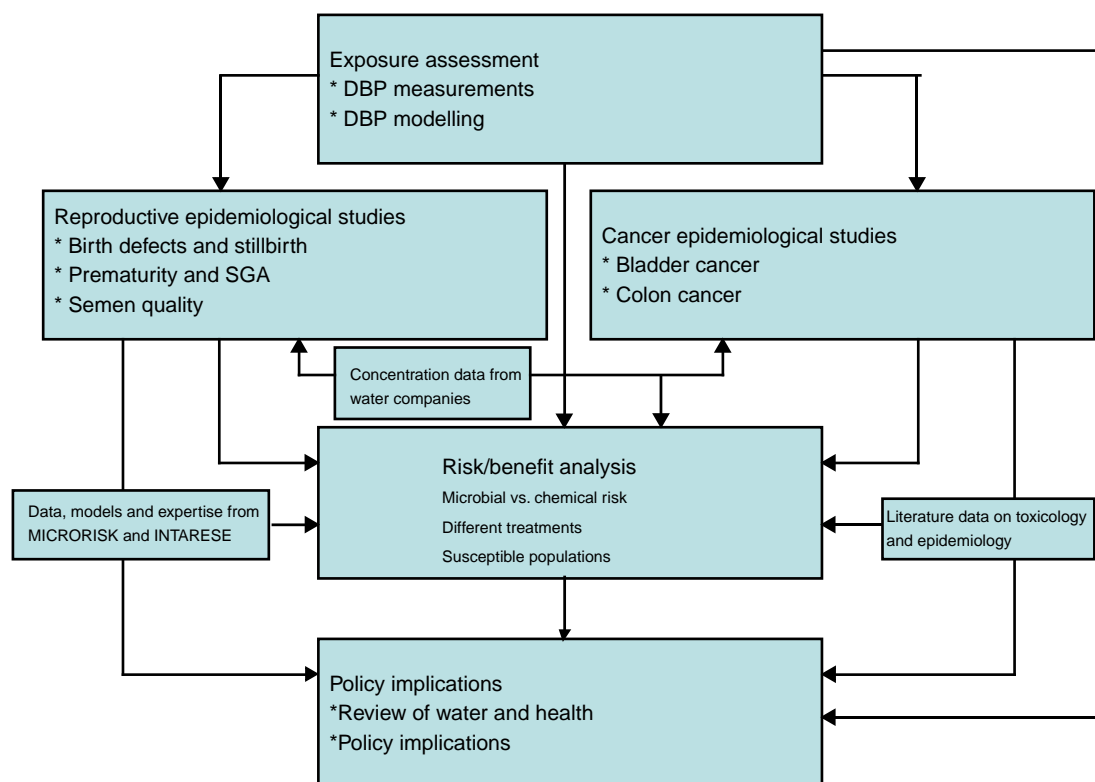


Figure 1 | Linkage of work and the application of the risk assessment work into policy.

current guideline values and treatment practices in Europe; and recommendations concerning EU legislation regarding the Water Framework Directive, Directive 98/83/EC, and other related directives. A conceptual model for application of the risk assessment work into policy is given in [Figure 1](#).

CONCLUSION

There appears to be very good epidemiological evidence for a relationship between chlorination by-products, as measured by THMs, in drinking water and bladder cancer, but the evidence for other cancers, including colorectal cancer appears to be inconclusive and inconsistent. There appears to be some evidence for a relationship between chlorination by-products, as measured by THMs, and small for gestational age (SGA)/intrauterine growth retardation (IUGR) and preterm delivery, but evidence for other outcomes such as low birth weight (LBW), stillbirth, congenital anomalies and semen quality appears to be inconclusive and inconsistent. Major limitations in exposure assessment may account for the inconclusive and inconsistent results in epidemiological studies, but there are other issues such as outcome definition and bias and confounding.

The HIWATE study brings together a number of leading researchers in Europe to carry out the research. A concerted European research effort has so far been lacking in this area, resulting in a widening gap of knowledge compared with North America. A larger number of people including scientists, policy-makers, industry and consumer representatives will meet during the proposed open workshop at the end of the project to produce European guidelines and recommendations and set a research agenda for further work.

Innovative aspects of the work include detailed exposure assessment methods in many of the studies taking into account not only the measurement of water levels of many by-products but also water-related activities/pathways such as ingestion, showering, bathing and swimming and routes (oral, skin absorption and inhalation) producing integrated exposure indices, particularly for THMs, but also other DBPs where relevant;

examination of gene–environment interaction and identification of genetically susceptible groups both in the epidemiological and risk–benefit studies, and pooling of studies across countries to increase the power of the studies. It will provide new risk estimates for various health outcomes in Europe, including not only cancer (specifically colon cancer) but also reproductive outcomes (specifically semen quality, foetal growth restriction, IUGR) and improved risk estimates for various other outcomes (congenital malformations, stillbirth, low birth weight) in relation to DBPs in the risk–benefit study. It will provide a framework and methodology to compare the microbial and chemical risks, particularly DBPs, and the risk–benefit study will include a range of DBPs rather than using, for example, only ‘chlorinated water’ or THMs. The gene–interaction studies may provide further insight into the mechanisms of action. For the first time there will be comparable data for a range of DBPs throughout various regions in Europe. The work is expected to finish in April 2010 and an international workshop is planned in London a few months before the end of the project (see www.hiwate.org for news).

ACKNOWLEDGEMENTS

The work is (partly) funded by HIWATE (www.hiwate.org). HIWATE is a three-and-a-half year *Specific Targeted Research Project*, funded under the EU Sixth Framework Programme for Research and Technological Development by the Research Directorate-Biotechnology, Agriculture and Food Research Unit (Contract no Food-CT-2006-036224). The HIWATE consortium consists of more participants than the current author list and we would like to thank them for their input.

REFERENCES

- Aggazzotti, G., Righi, E., Fantuzzi, G., Biasotti, B., Ravera, G., Kanitz, S., Barbone, F., Sansebastiano, E., Battaglia, M. A., Leoni, V., Fabiani, L., Triassi, M. & Sciacca, S. 2004 Collaborative group for the study of chlorinated drinking water and pregnancy chlorination by-products (CBPs) in drinking water and adverse pregnancy outcomes in Italy. *J. Water Health* 2, 233–247.

- Aschengrau, A., Zierler, S. & Cohen, A. 1993 Quantity of community drinking water and the occurrence of late adverse pregnancy outcomes. *Arch. Environ. Health* **48**, 105–113.
- Barbone, F., Valent, F., Brussi, V., Tomasella, L., Triassi, M., Scognamiglio, A., Righi, G., Fantuzzi, E., Casolari, G. & Aggazzotti, G. 2002 Assessing exposure to drinking water disinfection byproduct in pregnant women: validity and reproducibility of a questionnaire. *Epidemiology* **13**, 540–544.
- Bove, F. J., Fulcomer, M. C., Klotz, J. B., Esmart, J., Dufficy, E. M. & Savrin, J. E. 1995 Public drinking water contamination and birth outcomes. *Am. J. Epidemiol.* **141**, 850–862.
- Bove, F., Shim, Y. & Zeitz, P. 2002 Drinking water contaminants and adverse pregnancy outcomes: a review. *Environ. Health Perspect.* **110**(Suppl. 1), 61–74.
- Bove, G. E. Jr, Rogerson, P. A. & Vena, J. E. 2007 Case control study of the geographic variability of exposure to disinfectant byproducts and risk for rectal cancer. *Int. J. Health Geogr.* **6**, 18.
- Cantor, K. P., Villanueva, C., Garcia-Closas, M., Silverman, D., Real, F. X., Dosemeci, M., Malats, N., Yeager, M., Welch, R., Chanock, S., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castano-Vinyals, G., Samanic, C., Rothman, N. & Kogevinas, M. 2006 Bladder cancer, disinfection byproducts, and markers of genetic susceptibility in a case-control study from Spain. *Epidemiology* **17**(6), S150.
- Cedergren, M. I., Selbing, A. J., Lofman, O. & Kallen, B. 2002 Chlorination byproducts and nitrate in drinking water and risk of congenital cardiac defects. *Environ. Res. Section A* **89**, 124–130.
- Chen, J., Douglas, G. C., Thirkill, T. L., Lohstroh, P. N., Bielmeier, S. R., Narotsky, M. G., Best, D. S., Harrison, R. A., Natarajan, K., Pegram, R. A., Overstreet, J. W. & Lasley, B. L. 2003 Effect of bromodichloromethane on chorionic gonadotrophin secretion by human placental trophoblast cultures. *Toxicol. Sci.* **76**, 75–82.
- Chen, J., Thirkill, T. L., Lohstroh, P. N., Bielmeier, S. R., Narotsky, M. G., Best, D. S., Harrison, R. A., Natarajan, K., Pegram, R. A., Overstreet, J. W., Lasley, B. L. & Douglas, G. C. 2004 Bromodichloromethane inhibits human placental trophoblast differentiation. *Toxicol. Sci.* **78**, 166–174.
- Chevrier, C., Junod, B. & Cordier, S. 2004 Does ozonation of drinking water reduce the risk of bladder cancer? *Epidemiology* **15**(5), 605–614.
- Clyma, J.-A., Dippnall, M., Baillie, H., Povey, A., Cherry, N., Moore, H., McNamee, R., Pacey, A. & Burgess, G. 2008 Occupation and male infertility: glycol ethers and other exposures. *OEM* **65**(10), 708–714.
- Crider, K. S., Whitehead, N. & Buus, R. M. 2005 Genetic variation associated with preterm birth: a HuGE review. *Genet. Med.* **7**, 593–604.
- DeMarini, D. M., Shelton, M. L., Warren, S. H., Ross, T. M., Shim, J. Y., Richard, A. M. & Pegram, R. A. 1997 Glutathione S-transferase-mediated induction of GC!AT transitions by halomethanes in *Salmonella*. *Environ. Mol. Mutagen.* **30**, 440–447.
- Dodds, L. & King, W. D. 2001 Relation between trihalomethane compounds and birth defects. *Occup. Environ. Med.* **58**, 443–446.
- Dodds, L., King, W., Woolcott, C. & Pole, J. 1999 Trihalomethanes in public water supplies and adverse birth outcomes. *Epidemiology* **10**, 233–237.
- Dodds, L., King, W., Allen, A. C., Armson, A., Fell, D. B. & Nimrod, C. 2004 Trihalomethanes in public water supplies and risk of stillbirth. *Epidemiology* **15**, 179–186.
- Dojlido, J., Zbieć, E. & Świetlik, R. 1999 Formation of the haloacetic acids during ozonation and chlorination of water in Warsaw waterworks (Poland). *Water Res.* **33**, 3111–3118.
- Doria, M. F., Pidgeon, N. & Hunter, P. 2005 Perception of tap water risks and quality: a structural equation model approach. *Water Sci. Technol.* **52**(8), 143–149.
- Doria, M. F. 2006 Bottled water versus tap water: understanding consumers' preferences. *J. Water Health* **4**(2), 271–276.
- Dow, J. L. & Green, T. 2000 Trichloroethylene induced vitamin B12 and folate deficiency leads to increased formic acid excretion in the rat. *Toxicology* **146**, 123–136.
- Doyle, T. J., Zheng, W., Cerhan, J. R., Hong, C. P., Sellers, T. A., Kushi, L. H. & Folsom, A. R. 1997 The association of drinking water source and chlorination by-products with cancer incidence among postmenopausal women in Iowa: a prospective cohort study. *Am. J. Public Health* **87**, 1168–1176.
- Engel, S. A., Erichsen, H. C., Savitz, D. A., Thorp, J., Chanock, S. J. & Olshan, A. F. 2005a Risk of spontaneous preterm birth is associated with common proinflammatory cytokine polymorphisms. *Epidemiology* **16**, 469–477.
- Engel, S. A., Olshan, A. F., Savitz, D. A., Thorp, J., Erichsen, H. C. & Chanock, S. J. 2005b Risk of small-for-gestational age is associated with common anti-inflammatory cytokine polymorphisms. *Epidemiology* **16**, 478–486.
- Fantuzzi, G., Aggazzotti, G., Righi, E., Predieri, G., Giacobazzi, P., Kanitz, S., Barbone, F., Sansesebiano, G., Ricci, C., Leoni, V., Fabiani, L., Triassi, M. & Sciacca, S. and Collaborative Group for the Study of Chlorinated Drinking Waters and Pregnancy 2007 Valutazione dell'esposizione a composti organoalogenati nelle acque potabili di 9 province italiane: indagine sulla presenza di cloriti, clorati, trihalometani, trielina e tetracloroetilene. *Ann. Igiene* **19**, 345–354.
- Fenster, L., Waller, K., Windham, G., Henneman, T., Anderson, M., Mendola, P., Overstreet, J. W. & Swan, S. H. 2003 Trihalomethane levels in home tap water and semen quality. *Epidemiology* **14**, 650–658.
- Gallagher, M. D., Nuckols, J. R., Stallones, L. & Savitz, D. A. 1998 Exposure to trihalomethanes and adverse pregnancy outcomes. *Epidemiology* **9**, 484–489.
- Garcia-Closas, M., Malats, N., Silverman, D., Dosemeci, M., Kogevinas, M., Hein, D. W., Tardon, A., Serra, C., Carrato, A., Garcia-Closas, R., Lloreta, J., Castano-Vinyals, G., Yeager, M., Welch, R., Chanock, S., Chatterjee, N., Wacholder, S., Samanic, C., Tora, M., Fernandez, F., Real, F. X. & Rothman, N. 2005 NAT2 slow acetylation, GSTM1 null genotype,

- and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. *Lancet* **366**, 649–659.
- Gardosi, J. 2006 New definition of small for gestational age based on fetal growth potential. *Horm. Res.* **65**(suppl 3), 15–18.
- Gelbaya, T. A. & Nardo, L. G. 2005 Customised fetal growth chart: a systematic review. *J. Obstet. Gynaecol.* **25**(5), 445–450.
- Gemma, S., Vittozzi, L. & Testai, E. 2003 Metabolism of chloroform in the human liver and identification of the competent P450s. *Drug Metab. Dispos.* **31**, 266–274.
- Geter, D. R., Moore, T. M., George, M. H., Kilburn, S. R., Allen, J. W., Nelson, G. M., Winkfield, E. & DeAngelo, A. B. 2005 Tribromomethane exposure and dietary folate deficiency in the formation of aberrant crypt foci in the colons of F344/N rats. *Food Chem. Toxicol.* **43**, 1405–1412.
- Gevecker Graves, C., Matanoski, G. M. & Tarldiff, R. G. 2001 Weight of evidence for an association between adverse reproductive and developmental effects and exposure to disinfection by-products: a critical review. *Reg. Tox. Pharm.* **34**, 103–124.
- Golfinopoulos, S. K. & Arhonditsis, G. B. 2002 Multiple regression models: a methodology for evaluating trihalomethane concentrations in drinking water from raw water characteristics. *Chemosphere* **47**(9), 1007–1018.
- Golfinopoulos, S. K. & Nikolaou, A. D. 2005 Survey of disinfection by-products in drinking water in Athens, Greece. *Desalination* **176**(1–3), 13–24.
- Havelaar, A. H., De Hollander, A. E., Teunis, P. F., Evers, E. G., Van Kranen, H. J., Versteegh, J. F., Van Koten, J. E. & Slob, W. 2000 Balancing the risks and benefits of drinking water disinfection: disability adjusted life-years on the scale. *Environ. Health Perspect.* **108**(4), 315–321.
- Hildesheim, M. E., Cantor, K. P., Lynch, C. F., Dosemeci, M., Lubin, J., Alavanja, M. & Craun, G. 1998 Drinking water source and chlorination byproducts. II. Risk of colon and rectal cancers. *Epidemiology* **9**, 29–35.
- Hinckley, A. F., Bachand, A. M. & Reif, J. S. 2005 Late pregnancy exposures to disinfection by-products and growth-related birth outcomes. *Environ. Health Perspect.* **113**, 1808–1813.
- Hwang, B.-F. & Jaakkola, J. J. K. 2003 Water chlorination and birth defects: a systematic review and meta-analysis. *Arch. Environ. Health* **58**, 85–91.
- Hwang, B.-F., Magnus, P. & Jaakkola, J. J. K. 2002 Risk of specific birth defects in relation to chlorination and the amount of natural organic matter in the water supply. *Am. J. Epidemiol.* **156**, 374–382.
- Infante-Rivard, C. 2004 Drinking water contaminants, gene polymorphisms and fetal growth. *Environ. Health Perspect.* **112**, 1213–1216.
- IPCS 2000 Disinfectants and disinfectant by-products. *Environ. Health Criteria* **216**.
- Jaakkola, J. J. K., Magnus, P., Skrondal, A., Hwang, B.-F., Becher, G. & Dybing, E. 2001 Foetal growth and duration of gestation relative to water chlorination. *Occup. Environ. Med.* **58**, 437–442.
- Kallen, B. A. J. & Robert, E. 2000 Drinking water chlorination and delivery outcome: a registry-based study in Sweden. *Reprod. Toxicol.* **14**, 303–309.
- Kamen, B. 1997 Folate and antifolate pharmacology. *Semin. Oncol.* **24**, S18–30–S18–39.
- Kanitz, S., Franco, Y., Patrone, V., Caltabellotta, M., Raffo, E., Riggi, C., Timitilli, D. & Ravera, G. 1996 Association between drinking water disinfection and somatic parameters at birth. *Environ. Health Perspect.* **104**, 516–520.
- Karagas, M., Villanueva, C. M., Nieuwenhuijsen, M. J., Weisel, C. P., Cantor, C. P. & Kogevinas, M. 2008 Disinfection by-products and skin cancer: a hypothesis. *Cancer Cause Control* **19**(5), 547–548, doi:10.1007/s10552-008-9116-y.
- Kaur, S., Nieuwenhuijsen, M. J., Ferrier, H. & Steer, P. 2004 Exposure of pregnant women to tap water related activities in London and validation of the questionnaire. *Occup. Environ. Med.* **61**, 454–460.
- King, W. D., Marrett, L. D. & Woolcott, C. G. 2000 Case-control study of colon and rectal cancers and chlorination by-products in treated water. *Cancer Epidemiol. Biomarkers Prev.* **9**, 813–818.
- King, W. D., Dodds, L. & Allen, A. C. 2000 Relation between stillbirth and specific chlorination by-products in public water supplies. *Environ. Health Perspect.* **108**, 883–886.
- King, W. D., Dodds, L., Allen, A. C., Armson, B. A., Fell, D. & Nimrod, C. 2005 Haloaceticacids in drinking water and risk for stillbirth. *Occup. Environ. Med.* **62**, 124–127.
- Klotz, J. B. & Pynch, L. A. 1999 Neural tube defects and drinking water disinfection by-products. *Epidemiology* **10**, 383–390.
- Koivusalo, M. & Vartiainen, T. 1997 Drinking water chlorination by-products and cancer. *Rev. Environ. Health* **12**, 81–90.
- Koivusalo, M., Hakulinen, T., Vartiainen, T., Pukkala, E., Jaakkola, J. J. & Tuomisto, J. 1998 Drinking water mutagenicity and urinary tract cancers: a population-based case-control study in Finland. *Am. J. Epidemiol.* **148**, 704–712.
- Kramer, M. D., Lynch, C. F., Isacson, P. & Hanson, J. W. 1992 The association of waterborne chloroform with intrauterine growth retardation. *Epidemiology* **3**, 407–413.
- Krasner, S. W., Weinberg, H. S., Richardson, S. D., Pastor, S. J., Chinn, R., Scrimenti, M. J., Onstad, G. D. & Thruston, A. D., Jr 2006 Occurrence of a new generation of disinfection byproducts. *Environ. Sci. Technol.* **40**, 7112–7113.
- Larson, J. & Bull, R. 1992 Metabolism and lipid peroxidative activity of trichloroacetate and dichloroacetate in rats and mice. *Toxicol. Appl. Pharmacol.* **115**, 268–277.
- Lewis, C., Suffet, I. H. & Ritz, B. 2006 Estimated effects of disinfection by-products on birth weight in a population served by a single water utility. *Am. J. Epidemiol.* **163**, 38–47.
- Lewis, C., Suffet, I. H., Hoggatt, K. & Ritz, B. 2007 Estimated effects of disinfection by-products on preterm birth in a population served by a single water utility. *Environ. Health Perspect.* **115**, 290–295.

- Linder, R. E., Klinefelter, G. L., Strader, L. F., Suarez, J. D. & Dyer, C. J. 1994a Acute spermatogenic effects of bromoacetic acids. *Fund. Appl. Toxicol.* **22**, 422–430.
- Linder, R. E., Klinefelter, G. R., Strader, L. F., Suarez, J. D., Roberts, N. L. & Dyer, C. J. 1994b Spermatotoxicity of dibromoacetic acid in rats after 14 daily exposures. *Reprod. Toxicol.* **8**, 251–259.
- Linder, R. E., Klinefelter, G. R., Strader, L. F., Suarez, J. D., Roberts, N. L. & Perreault, S. D. 1995 Dibromoacetic acid affects reproductive competence and sperm quality in the male rat. *Fund. Appl. Toxicol.* **28**, 9–17.
- Linder, R. E., Klinefelter, G. R., Strader, L. F., Suarez, J. D. & Roberts, N. L. 1997a Spermatotoxicity of dichloroacetic acid. *Reprod. Toxicol.* **11**, 681–688.
- Linder, R. E., Klinefelter, G. R., Strader, L. F., Veeramachanemi, D. N., Roberts, N. L. & Suarez, J. D. 1997b Histopathologic changes in testes of rats exposed to dibromoacetic acid. *Reprod. Toxicol.* **11**, 47–56.
- Luben, T. J., Olshan, A. F., Herring, A. H., Jeffay, S., Strader, L., Buus, R. M., Chan, R. L., Savitz, D. A., Singer, P. C., Weinberg, H. S. & Perreault, S. D. 2007 The healthy men study: an evaluation of exposure to disinfection by-products in tap water and sperm quality. *Environ. Health Perspect.* **115**, 1169–1176.
- Magnus, P., Jaakkola, J. J. K., Skrandal, A., Alexander, J., Becher, G., Krogh, T. & Dybing, E. 1999 Water chlorination and birth defects. *Epidemiology* **10**, 513–517.
- Malliarou, M., Collins, C., Graham, N. & Nieuwenhuijsen, M. J. 2005 Haloacetic acids in drinking water in the UK. *Water Res.* **39**, 2722–2730.
- Meek, M. E., Beauchamp, R., Long, G., Moir, D., Turner, L. & Walker, M. 2002 Chloroform: exposure estimation, hazard characterization, and exposure-response analysis. *J. Toxicol. Environ. Health B Crit. Rev.* **5**, 283–334.
- Meriläinen, P., Lehtola, M., Grellier, J., Iszatt, N., Nieuwenhuijsen, M., Vartiainen, T. & Tuomisto, J. T. 2008 Developing a conceptual model for risk-benefit analysis of disinfection by-products and microbes in drinking water (in preparation).
- Min, J., Park, H., Park, B., Kim, Y. J., Park, J., Lee, H., Ha, E., Park, E. & Hong, Y. C. 2006 Paraoxonase gene polymorphism and vitamin levels during pregnancy: relationship with maternal oxidative stress and neonatal birth weights. *Reprod. Toxicol.* **22**, 418–424.
- Myatt, L. & Cui, X. 2004 Oxidative stress in the placenta. *Histochem. Cell Biol.* **122**, 369–382.
- Ni, Y. C., Wong, T. Y., Lloyd, R. V., Heinze, T. M., Shelton, S., Casciano, D., Kadlubar, F. F. & Fu, P. P. 1996 Mouse liver microsomal metabolism of chloral hydrate, trichloroacetic acid and trichloroethanol leading to induction of lipid peroxidation via a free radical mechanism. *Drug Metab. Dispos.* **24**, 81–90.
- Nieuwenhuijsen, M. J. (ed.) 2003 *Exposure Assessment in Occupational and Environmental Epidemiology*. Oxford University Press, Oxford.
- Nieuwenhuijsen, M. J., Toledano, M. B. & Elliott, P. 2000a Uptake of chlorination disinfection by-products; a review and a discussion of its implications for epidemiological studies. *J. Expo. Anal. Environ. Epidemiol.* **10**, 586–599.
- Nieuwenhuijsen, M. J., Toledano, M. B., Eaton, N. E., Elliott, P. & Fawell, J. 2000b Chlorination disinfection by-products in water and their association with adverse reproductive outcomes: a review. *Occup. Environ. Med.* **57**, 73–85.
- Nieuwenhuijsen, M. J., Toledano, M. B., Bennett, J., Best, N., Konstantinou, K., Hambly, P., de Hoogh, K., Boyd, P., Abramsky, L., Wellesley, D., Dattani, N., Briggs, D., Lars Jarup, L. & Elliott, P. 2008 The relationship between disinfection byproducts in drinking water and congenital anomalies in England and Wales. *Environ. Health Perspect.* **116**, 216–222.
- Nikolaou, A. D., Golfinopoulos, S. K., Arhonditsis, G. B., Kolovoyiannis, V. & Lekkas, T. D. 2004 Modeling the formation of chlorination by-products in river waters with different quality. *Chemosphere* **55**, 409–420.
- Nissinen, T. K., Miettinen, I. T., Martikainen, P. J. & Vartiainen, T. 2002 Disinfection by-products in Finnish drinking waters. *Chemosphere* **48**, 9–20.
- Palacios, M., Pampillon, J. F. & Rodriguez, M. E. 2000 Organohalogenated compounds levels in chlorinated drinking waters and current compliance with quality standards throughout the European Union. *Water Res.* **34**, 1002–1016.
- Pegram, R. A., Andersen, M. E., Warren, S. H., Ross, T. M. & Claxton, L. D. 1997 Glutathione S-transferase-mediated mutagenicity of trihalomethanes in *Salmonella typhimurium*: contrasting results with bromodichloromethane and chloroform. *Toxicol. Appl. Pharmacol.* **144**, 183–188.
- Persson, F., Långmark, J., Heinicke, G., Hedberg, T., Tobiasson, J., Stenström, T. A. & Hermansson, M. 2005 Characterisation of the behaviour of particles in biofilters for pre-treatment of drinking water. *Wat. Res.* **39**(16), 3791–3800.
- Peters, J. L., Rushton, L., Sutton, A. J., Jones, D. R., Abrams, K. R. & Mugglestone, M. A. 2005 Bayesian methods for the cross-design synthesis of epidemiological and toxicological evidence. *Appl. Stat.* **54**(1), 159–172.
- Porter, C. K., Putnam, S. D., Hunting, K. L. & Riddle, M. R. 2005 The effect of trihalomethane and haloacetic acid exposure on fetal growth in Maryland county. *Am. J. Epidemiol.* **162**, 334–344.
- Potter, C. L., Chang, L. W., DeAngelo, A. B. & Daniel, F. B. 1996 Effects of four trihalomethanes on DNA strand breaks, renal hyaline droplet formation and serum testosterone in male F-344 rats. *Cancer Lett.* **106**, 235–242.
- Ramon, R., Ballester, F., Rebagliato, M., Ribas, N., Torrent, M., Fernandez, M., Sala, M., Tardon, A., Marco, A., Posada, M., Grimalt, J. & Sunyer, J. 2005 Red INMA. The Environment and Childhood Research Network (“INMA” network): study protocol. *Rev. Esp. Salud Publica.* **79**(2), 203–220.
- Ray, J. G. & Laskin, C. A. 1999 Folic acid and homocyst(e)ine metabolic defects and the risk of placental abruption, pre-eclampsia and spontaneous pregnancy loss: a systematic review. *Placenta* **20**, 519–529.
- Reif, J. S., Hatch, M. C., Bracken, M., Holmes, L. B., Schwetz, B. A. & Singer, P. C. 1996 Reproductive and development effects of disinfection by-products in drinking water. *Environ. Health Perspect.* **104**, 1056–1061.

- Richardson, S. 1998 Drinking water disinfection by-products. In *Encyclopedia of Environmental Analysis and Remediation* (R. A., Meyers, ed.), pp. 1398–1421. John Wiley & Sons, New York.
- Richardson, S. D., Plewa, M. J., Wagner, E. D., Schoeny, R. & DeMarini, D. M. 2008 Occurrence, genotoxicity, and carcinogenicity of emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat. Res. Rev. Mutat. Res.* doi:10.1016/j.mrrev.2007.09.001.
- Rook, J. J. 1974 Formation of haloforms during chlorination of natural waters. *J. Soc. Water Treat. Exam.* **23**, 234–243.
- Ross, M. K. & Pegram, R. A. 2004 In vitro biotransformation and genotoxicity of the drinking water disinfection byproduct bromodichloromethane: DNA binding mediated by glutathione transferase theta 1-1. *Toxicol. Appl. Pharmacol.* **195**, 166–181.
- Sadiq, R. & Rodriguez, M. 2004 Disinfection by-products (DBPs) in drinking water and predictive models for their occurrence: a review. *Sci. Total Environ.* **321**(1–3), 21–46.
- Savitz, D. A., Andrews, K. W. & Pastore, L. M. 1995 Drinking water and pregnancy outcome in Central North Carolina: Source, amount and trihalomethane levels. *Environ. Health Perspect.* **103**, 592–596.
- Savitz, D. A., Singer, P. C., Herring, A. H., Hartmann, K. E., Howard, S., Weinberg, H. S. & Makarushka, C. 2006 Exposure to drinking water disinfection by-products and pregnancy loss. *Am. J. Epidemiol.* **164**, 1043–1051.
- Scholl, T. O. & Stein, T. P. 2001 Oxidant damage to DNA and pregnancy outcome. *J. Matern. Fetal Med.* **10**, 182–185.
- Sciuto, A. M., Clapp, D. L., Hess, Z. A. & Moran, T. S. 2003 The temporal profile of cytokines in the bronchoalveolar lavage fluid in mice exposed to the industrial gas phosgene. *Inhal. Toxicol.* **15**, 687–700.
- Shaw, G. M., Ranatunga, D., Quach, T., Neri, E., Correa, A. & Neutra, R. 2003 Trihalomethane exposures from municipal water supplies and selected congenital malformations. *Epidemiology* **14**, 191–199.
- Smith, M. K., Randall, J. L. & Stober, J. A. 1989 Teratogenic activity of trichloroacetic acid in the rat. *Teratology* **40**, 445–451.
- Spiegelhalter, D. J. 2003 *WinBUGS: Bayesian Inference Using Gibbs Sampling Manual Version 1.4*. Imperial College, London and MRC Biostatistics Unit, Cambridge.
- Tardiff, R. G., Carson, M. L. & Ginevan, M. E. 2006 Updated weight of evidence for an association between adverse reproductive and developmental effects and exposure to disinfection by-products. *Regul. Toxicol. Pharmacol.* **45**, 185–205.
- Toledano, M. B., Nieuwenhuijsen, M. J., Best, N., Whitaker, H., Hambly, P., de Hoogh, C., Fawell, J., Jarup, L. & Elliott, P. 2005 Relation of trihalomethane concentration in public water supplies to stillbirth and birth weight in three water regions in England. *Environ. Health Perspect.* **113**, 225–232.
- Tomasi, A., Albano, E., Biasi, F., Slater, T., Vannini, V. & Dianzani, M. U. 1985 Activation of chloroform and related trihalomethanes to free radical intermediates in isolated hepatocytes and in the rat in vivo as detected by the ESP-Spin trapping technique. *Chem.-Biol. Interact.* **55**, 303–316.
- Toth, G. P., Kelty, K. C., George, E. L., Read, E. J. & Smith, M. K. 1992 Adverse male reproductive effects following subchronic exposure of rats to sodium dichloroacetate. *Fund. Appl. Toxicol.* **19**, 57–63.
- Tuomisto, J. T. & Pohjola, M. (eds) 2007 *Open Risk Assessment. A New Way of Providing Scientific Information for Decision-making*. Kansanterveyslaitoksen julkaisu B18, Finland.
- Villanueva, C. M., Grimalt, J. O. & Kogevinas, M. 2003 Haloacetic acids and trihalomethane concentrations in finished drinking waters from different sources. *Water Res.* **37**(4), 954–959.
- Villanueva, C. M., Cantor, K. P., Cordier, S., Jaakkola, J. J., King, W. D., Lynch, C. F., Porru, S. & Kogevinas, M. 2004 Disinfection byproducts and bladder cancer: a pooled analysis. *Epidemiology* **15**, 357–367.
- Villanueva, C. M., Cantor, K. P., Grimalt, J. O., Castano-Vinyals, G., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Rothman, N., Real, F. X., Dosemeci, M. & Kogevinas, M. 2006 Assessment of lifetime exposure to trihalomethanes through different routes. *Occup. Environ. Med.* **63**(4), 273–277.
- Villanueva, C. M., Cantor, K. P., Grimalt, J. O., Malats, N., Silverman, D., Tardon, A., Garcia-Closas, R., Serra, C., Carrato, A., Castaño-Vinyals, G., Marcos, R., Rothman, N., Real, F. X., Dosemeci, M. & Kogevinas, M. 2007 Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering and swimming in pools. *Am. J. Epidemiol.* **165**, 148–156.
- Waller, K., Swan, S. H., DeLorenze, G. & Hopkins, B. 1998 Trihalomethanes in drinking water and spontaneous abortion. *Epidemiology* **9**, 134–140.
- Waller, K., Swan, S., Windham, G. C. & Fenster, L. 2001 Influence of exposure assessment methods on risk estimates in an epidemiologic study of trihalomethane exposure and spontaneous abortion. *J. Expo. Anal. Environ. Epidemiol.* **11**, 522–531.
- Weber, L. W., Boll, M. & Stampfl, A. 2003 Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.* **33**, 105–136.
- Weinberg, H. S., Krasner, S. W., Richardson, S. D. & Thruston, A. D. 2002 *The Occurrence Of Disinfection By-Products (DBPs) of Health Concern in Drinking Water: Results of a Nationwide DBP Occurrence Study*. EPA report: EPA 600/R-02/068 (http://www.epa.gov/athens/publications/reports/EPA_600_R02_068.pdf).
- Westrell, T., Bergstedt, O., Stenström, T.-A. & Ashbolt, N. 2003 A theoretical approach to assess microbial risks due to failures in drinking water systems. *Int. J. Environ. Health* **13**(2), 181–197.
- Westrell, T., Schonning, C., Stenstrom, T. A. & Ashbolt, N. J. 2004 QMRA (quantitative microbial risk assessment) and HACCP (hazard analysis and critical control points) for management of pathogens in wastewater and sewage sludge treatment and reuse. *Water Sci. Technol.* **50**(2), 23–30.

- Westrell, T., Andersson, Y. & Stenström, T. A. 2006 Drinking water consumption patterns in Sweden. *J. Water Health* **4**(4), 511–522.
- Whitaker, H., Nieuwenhuijsen, M. J. & Best, N. 2003 The relationship between water chloroform levels and uptake of chloroform: a simulation study. *Environ. Health Perspect.* **111**, 688–694.
- Whitaker, H., Best, N., Nieuwenhuijsen, M. J., Wakefield, J., Fawell, J. & Elliott, P. 2005 Modelling exposure to disinfection by-products in drinking water for an epidemiological study of adverse birth outcomes. *J. Expo. Anal. Environ. Epidemiol.* **15**, 138–146.
- Wilcox, A. J. 2001 On the importance—and the unimportance—of birthweight. *Int. J. Epidemiol.* **30**, 1233–1241.
- Wilkins, J. R. III & Comstock, G. W. 1981 Source of drinking water at home and site-specific cancer incidence in Washington County, Maryland. *Am. J. Epidemiol.* **114**, 178–190.
- Wright, J. M., Schwartz, J. & Dockery, D. W. 2003 Effect of trihalomethane exposure on fetal development. *Occup. Environ. Med.* **60**, 173–180.
- Wright, J. M., Schwartz, J. & Dockery, D. W. 2004 The effect of disinfection by-products and mutagenic activity on birth weight and gestational duration. *Environ. Health Perspect.* **112**, 920–925.
- Yang, C.-Y. 2004 Drinking water chlorination and adverse birth outcomes in Taiwan. *Toxicology* **198**, 249–254.
- Yang, C.-Y., Cheng, B.-H., Tsai, S.-S., Wu, T.-N., Lin, M.-C. & Lin, K.-C. 2000 Association between chlorination of drinking water and adverse pregnancy outcome in Taiwan. *Environ. Health Perspect.* **108**, 765–768.
- Yang, C. Y., Xiao, Z. P., Ho, S. C., Wu, T. N. & Tsai, S. S. 2007 Association between trihalomethane concentrations in drinking water and adverse pregnancy outcome in Taiwan. *Environ. Res.* **104**(3), 390–395.
- Young, T. B., Kanarek, M. S. & Tsiatis, A. A. 1981 Epidemiologic study of drinking water chlorination and Wisconsin female cancer mortality. *J. Natl Cancer Inst.* **67**, 1191–1198.
- Young, T. B., Wolf, D. A. & Kanarek, M. S. 1987 Case-control study of colon cancer and drinking water trihalomethanes in Wisconsin. *Int. J. Epidemiol.* **16**, 190–197.

First received 2 April 2008; accepted in revised form 26 July 2008. Available online February 2009