

**Research paper** for Environmental Health, August 24, 2007

Water Disinfection By-products and the Risk of Specific Birth Defects:  
A Population-based Cross-Sectional Study in Taiwan

Bing-Fang Hwang,<sup>1,2,3</sup> Jouni J.K. Jaakkola,<sup>4</sup> How-Ran Guo<sup>2</sup>

## Affiliations

<sup>1</sup> Department and Graduate Institute of Occupational Safety and Health, College of Public Health, China Medical University, Taichung, Taiwan.

<sup>2</sup> Department of Occupational and Environmental Medicine, National Chung Kung University, Tainan, Taiwan.

<sup>3</sup> Department of Health Care Administration, Diwan College of Management, Tainan, Taiwan.

<sup>4</sup> The Institute of Occupational and Environmental Medicine, The University of Birmingham, Birmingham, UK.

### E-mail address:

Bing-Fang Hwang ([bfhwang@mail.cmu.edu.tw](mailto:bfhwang@mail.cmu.edu.tw))

Jouni JK Jaakkola ([j.jaakkola@bham.ac.uk](mailto:j.jaakkola@bham.ac.uk))

How-Ran Guo ([hrguo@mail.ncku.edu.tw](mailto:hrguo@mail.ncku.edu.tw))

### Correspondence with:

Dr. Jouni JK Jaakkola,

The Institute of Occupational and Environmental Medicine,

The University of Birmingham, Birmingham, UK.

Tel: 0121 414 6023

Fax: 0121 414 6217

Email: [J.Jaakkola@bham.ac.uk](mailto:J.Jaakkola@bham.ac.uk)

## Abstract

**Background:** Recent findings suggest that exposure to disinfection by-products may increase the risk of birth defects. Previous studies have focused mainly on birth defects in general or groups of defects. The objective of the present study was to assess the effect of water disinfection by-products on the risk of most common specific birth defects.

**Method:** We conducted a population-based cross-sectional study of 396,049 Taiwanese births in 2001-2003 using information from the Birth Registry and Waterworks Registry. We compared the risk of nine most common specific defects in four disinfection by-product exposure categories based on the levels of total trihalomethanes (TTHMs) representing high (TTHMs 20+  $\mu\text{g/L}$ ), medium (TTHMs 10-19  $\mu\text{g/L}$ ), low exposure (TTHMs 5-9  $\mu\text{g/L}$ ), and 0-4  $\mu\text{g/L}$  as the reference category. In addition, we conducted a meta-analysis of the results from present and previous studies focusing on the same birth defects.

**Results:** In multivariate logistic regression analysis, the risk of ventricular septal defects (adjusted odds ratio 1.81, 95% confidence interval 0.98-3.35), cleft lip (1.56, 1.00-2.41), and anencephalus (1.96, 0.94-4.07) were elevated in the high exposure compared to the reference category. In the meta-analysis, the summary odds ratios for ventricular septal defect (1.81, 1.21-2.71) and cleft lip (1.66, 1.15-2.40) were consistently elevated.

**Conclusions:** The weight of evidence from the present study and previous epidemiologic studies suggests that prenatal exposure to disinfection by-products increases the risk of ventricular septal defects and cleft lip.

**KEYWORDS:** birth defects; water disinfection; disinfection by-products; total trihalomethanes

Running Head: Water disinfection and abnormalities

Text: 3057 words; Abstract: 231 words; 5 tables; 23 references

## Background

Water chlorination is a widely used and efficient method to reduce the occurrence of water-borne diseases, and has been one of the most successful public health measures introduced in the 20<sup>th</sup> century. In early 1970s, some volatile halogenated organic compounds, such as chloroform, were identified in chlorinated surface waters containing high levels of natural organic material.[1] Later many other disinfection by-products, such as other trihalomethanes (THMs), haloacetic acids, chlorophenols, chloral hydrate, and haloacetonitriles have been identified, most from the process of chlorination, but also from chloramination, chlorine dioxide disinfection, and ozonation.[2] Generally, the THMs, including chloroform, bromodichloromethane, dibromochloromethane and bromoform, are the most prevalent in chlorinated surface water.[3] They are routinely measured throughout water works in Taiwan.

A recent meta-analysis of the 5 studies published by the end of 2001 indicated that exposure to chlorination by-products may increase the risk of birth defects in general, especially neural tube and urinary tract defects.[4-9] More recently a Swedish study provided evidence of an elevated risk of cardiac defects,[10] whereas two Californian case-control studies of neural tube defects, cleft lip and cleft palate provided inconsistent results.[11] In a Norwegian nationwide cross-sectional study the risk of ventricular septal defect, cleft lip and obstructive urinary tract defects were related to exposure to disinfection by products.[12]

We conducted a cross-sectional study of all Taiwanese births in years 2001-2003 to assess the effect of water disinfection by-products on the most common specific birth defects. We also synthesized quantitatively our results with those of the Norwegian study for the birth defects available for both studies.

## **Methods**

### **Study Population**

The source population comprised of all 721,289 newborns registered by the Taiwanese Birth Registry from 2001 to 2003. We excluded 325,240 newborns due to insufficient information on disinfection practice in the municipality where the mother was living during pregnancy. We focused on five water regions, which were served by only one type of waterworks. The final study population included 396,049 infants. The study protocol was approved by the Institutional Review Board of Diwan College of Management, and it complied with the principles outlined in the Helsinki Declaration.[13]

### **Health Outcomes.**

We assessed the effect of disinfection by-products on the risks of the nine most common specific birth defects according to the International Classification of Diseases, ninth revision (ICD-9), including anencephalus (740.0), hydrocephalus (741.0), ventricular septal defects (745.4), atrial septal defects (745.5), Tetralogy of Fallot (745.2), cleft palate (749.0), cleft lip (749.1), renal agenesis & dysgenesis (753.0) and hypospadias (752.61).

All births within 15 days are compulsorily reported to the Taiwan Local Household Registry, which is managed by both the Department of Interior Affairs and the Department of Health. During the child's first week of life, a physician, most often a paediatrician makes diagnoses of the birth defects to be reported. As a result, birth defects diagnosed later in life are not included in the registry. According to ICD-9, up to three birth defects are coded for each child. We included all birth defects with ICD-9 codes 740.0-759.9. The following diagnoses were excluded due to low reliability [8]: other anomalies of nose (748.1), congenital hydrocele testis (752.4), clubfoot (754.0-754.8), congenital dislocation of the hip

(754.3), other specified anomalies of muscle, tendon, and fascia (756.8), and unspecified congenital anomalies (759.9).

### **Exposure Assessment**

Assessment of exposure was based on municipal-level water quality information on concentrations of total trihalomethanes (TTHMs), and mothers' place of residence during pregnancy. One or more waterworks serve each municipality and the waterworks seldom serve across the municipality borders. The Taiwanese water supply system is quite simple. Two hundred waterworks from Taiwanese Water Supply Corporation (TWSC) serving about 21 million people (90%) chlorinate their water, and privately owned wells (groundwater) serving about 2 million people (10%) do not use chlorination.

The general hypothesis was that exposure to disinfection by-products through tap water increases the risk of birth defects. We divided waterworks by the use of total trihalomethanes (TTHMs) (in  $\mu\text{g/L}$ ) as a quantitative measure of the water disinfection by-products. The TTHM level is recorded routinely in most of the waterworks. Under the regulations operating during the study period, the standard sampling frequency for THMs was a minimum of four samples per year. We assessed exposure by calculating a weighted average of the modeled quarterly TTHM estimates for the appropriate waterworks for the date of conception before the date of birth. The weighting was based on the proportion of the trimester falling into each quarterly period. We focused on five water regions where waterworks were served by only one type of water chlorination. In general, these regions reported similar overall prevalence of any birth defect.

## **Covariates**

We used routine birth registry data to construct the following covariates: gender of infant (male; female), maternal age (<20 years; 20-34 years;  $\geq$ 35 years), plurality (singleton; and multiple birth), and mother's health status including diabetes mellitus, and high fever. Maternal high fever was defined as the body temperature of pregnant woman after delivery greater than 38 °C We received municipal level data from the Department of Household Registration Affairs, Taiwanese Population Data services, which were used to construct municipal level population density, which is a measure of the proportion of urban population in the municipality.

## **Statistical Methods**

We estimated the prevalence (%) of the birth defects with 95% confidence intervals based on binomial distribution. We compared the risk of birth defects in three exposure categories (TTHMs 20+  $\mu\text{g/L}$ ; TTHMs 10-19  $\mu\text{g/L}$ ; TTHMs 5-9  $\mu\text{g/L}$ ) to the reference category with the lowest concentrations of TTHMs (0-4  $\mu\text{g/L}$ ). We used prevalence odds ratio as a measure of association and we applied logistic regression to estimate the adjusted odds ratios. The goodness of fit was tested with likelihood ratio tests (LR) to assess whether or not a variable contributes significantly to the model. First, we fitted a full model with a complete set of covariates. To elaborate sources of confounding, we fitted models with different combinations of covariates and compared the effect estimates from models with and without the covariate of interest. If the adjusted results differed from unadjusted results by  $> 10\%$ , the variable was included in the model. To evaluate the potential role of residual confounding and effect modification, we systematically compared effect estimates on different levels of covariates.

Finally, we conducted a meta-analysis of the present and the other available Norwegian study of specific birth defects in relation to water chlorination.[12] We calculated summary odds ratios using both the fixed-effects and random-effects models. The fixed-effects model was calculated using the Mantel-Haenszel method with inverse variances of individual effect estimates as weights.[14] The random-effects models were calculated using the method of DerSimonian and Laird.[15] We calculated summary odds ratios using the estimates from the contrast between the highest and the reference category. We studied heterogeneity of the independent studies by plotting the measures of effect and applying Q statistic. We elaborated the heterogeneity between the specific effect estimates, but presented systematically summary estimates from both fixed and random-effects models to offer readers a possibility for their own informed judgment.

## Results

Among 369,049 newborns in the study population, we identified 2,148 births (0.5%) with one or several birth defects of interest. Table 1 displays the study population according to the exposure categories. The municipalities in the high exposure category had a lower population density compared with the reference municipalities. Table 2 shows a comparison of the populations included and excluded from the analyses, and the total population. There were no statistically significant differences ( $\chi^2$  test;  $p > 0.05$ ) between the characteristics of the included population and the total population.

Table 3 shows that the prevalence of any birth defect was not directly related to the level of exposure. Further, the risks of anencephalus (adjusted OR 1.96, 95% CI 0.94-4.07), ventricular septal defects (1.81, 0.98-3.35), and cleft lip (1.56, 1.00-2.41) were substantially higher in the high exposure category compared with the reference category. The effect estimate for renal agenesis and dysgenesis was slightly elevated (1.27, 0.69-2.33). The exposure-response pattern for anencephalus was inconsistent with an elevated effect estimate in low but not in medium exposure category. The risk of ventricular septal defect was elevated only in the high exposure category, whereas the risk of cleft lip showed an exposure-response pattern.

Table 4 shows the characteristics of the present study and four previous studies and gives the study-specific adjusted odds ratios for the available outcomes. In four of the studies chlorine was used in disinfection, whereas in the Swedish study chlorine dioxide was applied.

In table 5, the summary odds ratios for ventricular septal defects (summary OR 1.81, 95% CI 1.21-2.71) and cleft lip (summary OR 1.66, 95% CI 1.15-2.40) provided consistent evidence of an increased risk, whereas the summary odds ratios for atrial septal defect and cleft palate provided consistent evidence of no effect. The summary odds ratio for hydrocephalus, based on three studies, was slightly elevated (random effects model: summary

OR 1.21, 95% CI 0.52-2.81, heterogeneity:  $p=0.152$ ) but showed some heterogeneity. The effect estimate for anencephalus (fixed effects model: summary OR 1.13, 95% CI 0.74-1.73, heterogeneity:  $p=0.056$ ) was slightly elevated but the study-specific effect estimates were heterogeneous. The sensitivity analysis revealed that one of the Californian case-control studies was responsible for the heterogeneity. After exclusion of this study the summary odds ratio was homogeneous and substantially elevated (fixed effects model: 1.73 (1.01-2.96, heterogeneity:  $p=0.619$ ).

## **Discussion**

Assessment of potential effects of exposure to disinfection by-products on the risk of specific birth defects is problematic because of rarity and diversity of the congenital malformations. Few previous studies have focused on selected birth defects and only one has assessed the associations between exposure and the risk of all the most common specific birth defects. [12]

Our results showed no consistent association between exposure and the risk of birth defects in general. Of the 9 specific birth defects studied, the risk of ventricular septal defects, cleft lip, and anencephalus were substantially elevated in the high exposure compared to the reference category. The meta-analysis of 6 birth defects together with the Norwegian and California data strengthened the evidence for ventricular septal defects and cleft lip.

## **Validity of Results**

The present study had enough power to estimate the relations between exposure to disinfection by-products and the most common specific birth defects, which could be relevant adverse effects based on previous literature. The meta-analysis together with the Norwegian and California results improved the precision of the two main findings. The specific birth defects are expected to be also more homogenous as to their causal factors compared with

birth defect groups. We excluded half of the births because of insufficient water quality data. This exclusion was unlikely to introduce selection bias, because it was made on municipality level and the characteristic of the excluded individuals did not differ substantially from the included, as shown in Table 2.

Our outcome assessment was based on birth registration, as in the vast majority of the previous studies of disinfection by-products and birth defects, [5,6,7,8,10,12] rather than clinical examination for the purposes of the study. This is a source of misclassification, which is likely to be random, i.e. not related to the exposure of interest, and thus lead to underestimation of the effect estimates. The sources of misclassification could include variation in diagnostic criteria, and errors reporting information provided by physician or hospital. Important features in the Taiwan national health care system limit the amount of outcome misclassification. Taiwanese pregnant women are almost all covered by health insurance (>99%) and access to prenatal care is free and good (at least 10 times during pregnancy). The follow-up time is from 1 month after conception through 7 days after birth. Birth defects were mostly diagnosed by physician, most often by paediatrician using ultrasound. Further, a study of validation of the Taiwanese birth registration using obstetric records showed a low rate of missing information (1.6%) and high levels of validity (sensitivity and specificity was 92.8%, and 99.6% respectively) and reliability (Cohen's  $\kappa$  statistics was 0.92). [16] According to the law, all live births in Taiwan must be reported within 15 days after birth. In general, the birth defects might be underreported, because we did not include the birth defects that were diagnosed after 7 days of age, gestational age less than 20 weeks and induced abortion due to birth defects. However, in the presence of a true association, random underreporting would weaken the observed association rather than introduce a spurious effect.

A major challenge of this study was the imprecision of exposure assessment from using aggregate municipal measures for classifying individual exposures. We had no information on the amounts of beverage and tap water consumption by pregnant women and exposure to volatile disinfection by-products through inhalation and dermal absorption, which might introduce non-differential misclassification and decrease the accuracy of exposure assessment. Future exposure assessment should include exposure through multiple routes such as bathing, showering and swimming, as well as water consumption. Unfortunately, we don't have sufficient information on alcohol consumption, cigarette smoking, vitamin consumption, medication, and genetic factors. Adjustment for population density not only adjusted indirectly for municipal differences in these behavior factors, but also eliminated partly underreporting bias between regions. We systematically carried out stratified analyses in different categories of exposure and other covariates to elaborate the residual confounding and potential effect modification. The stratified analyses did not indicate any major confounding or effect modification.

In a previous Norwegian study of the effects of water chlorination, six out of nine birth defects were the same as in the present study which made a possibility of meta-analysis appealing. Although the exposure assessment in the Norwegian study was based on the amount of organic content expressed in colour and the presence of chlorination, it was possible to use a similar contrast between the highest exposure category and the reference group of no exposure. Both studies adjusted for a number of individual and municipal level confounders. The set of confounders were different and therefore a meta-analysis was preferred to a pooled analysis of data.

## Synthesis with Previous Knowledge

Six [5,6,7,8,11,17] of the eight previous studies[5,6,7,8,10,11,12,17] have provided evidence of an increase in the risk of neural tube defects related to exposure to disinfection by-products. Two large case-control studies in California focusing on anencephalus and spina bifida did not provide a clear pattern of the relation of exposure to THMs.[11] A case-control study in New Jersey reported no significant association between exposure to THMs and the risk of spina bifida.[17] Interestingly in the present study the risk of anencephalus was elevated in the highest exposure category. The meta-analysis of the four available studies provided an inconclusive, heterogeneous summary estimate.

The previous findings on cardiac defects as a group have been heterogeneous and inconsistent.[5,6,8,10,12] In the present study, the risk of ventricular septal defects was almost two times higher in the high exposure category compared to the reference category (adjusted OR 1.81, 95% CI 0.98-3.35). This is consistent with the Norwegian study, which reported an exposure-related increase of ventricular septal defect (medium exposure: adjusted OR 1.63, 95% CI 1.02-2.58; high exposure 1.81, 1.05-3.09). The corresponding summary OR was 1.81 (95% CI 1.21-2.71). Recently the risk of ventricular septal defects has also been found to be related to the level of traffic-related air pollution. [19]

Previous five studies [5,6,8,11,12] on cleft lip and palate defects have given heterogeneous and/or inconsistent results. In the present study, the risk of cleft lip was related to the levels of THMs with an exposure-response pattern, yielding adjusted OR of 1.17 (95% CI: 0.74-1.86) for medium and 1.56 (95% CI: 1.00-2.41) for highest exposure category. Similar results were reported also from the Norwegian and California studies [9,11]. The three studies gave the summary odds ratio of 1.66 (95% CI: 1.15-2.40).

Previous studies conducted in Massachusetts [4] and Norway [8,12] have provided rather homogeneous and consistent evidence of an effect on urinary tract defects as a group.

In the present study there was no association between exposure and urinary tract defects per se, a smaller group denoted as renal agenesis and dysgenesis (ICD-9: 753.0) was found to be slightly elevated both in the high and low exposure categories. The Norwegian study showed some evidence of an increased risk of urinary tract defects as a group and an exposure response pattern for the risk of obstructive urinary tract defects (ICD-8: 753.2). [12] Urinary tract defects are rare and the effect estimate imprecise, and therefore the findings of the role of exposure to disinfection by-products remain inconclusive.

Our study and a recent Norwegian study suggest that prenatal exposure to disinfection by-products increases the risk of ventricular septal defects at much lower levels than United States, [5,7] United Kingdom,[20] and Canadian[6] drinking water sources, probably explained by qualitative geographic differences in the levels of natural organic matter (disinfection by-products precursor) or higher concentration of other non-volatile disinfection by-products (eg. haloacetic acids). The present and the two previous studies from Norway and California suggest also an increased risk of cleft lip.

### **Biologic Mechanisms**

The specific mechanisms for the effects of trihalomethanes (THMs) on the risk birth defects are still unknown. Some animal studies show reproductive and developmental toxicity of some of these compounds, such as chloroform (CF), and bromodichloromethanes (BDCM), when given at high doses.[3] There is evidence that metabolites of chloroform may accumulate in the amniotic fluid of pregnant mice.[21] In addition, BDCM can disrupt syncytiotrophoblast formation and inhibit chorionic gonadotrophin secretion in vitro.[22] This implies that the placenta is a likely target of BDCM toxicity in human and thus BDCM may have teratogenic effects on fetus.

An alternative explanation is that THMs may lead to birth defects via genetic damage to maternal gametes. For example, CF may be oxidatively metabolized and decomposed to electrophilic phosgene, which is more likely to react and bind to cell components including proteins, phospholipid polar heads, and reduced glutathione.[23] This may result in chromosomal abnormalities, enzymatic malfunction and disruption of cellular membranes, all of which could interfere with uterine development or directly influence on the conceptus.

## **Conclusions**

The present study suggests that prenatal exposure to disinfection by-products increases the risk of ventricular septal defects, cleft lip, and anencephalus. The findings on ventricular septal defect and cleft lip are consistent with previous epidemiologic studies [11, 12], which strengthens the weight of evidence on teratogenic effects of THMs. Our findings also demonstrate the importance of focusing on specific birth defects, rather than using broad categories of outcomes based on organ systems.

**Abbreviations**

CI = confidence interval

TTHMs = total trihalomethanes

ICD-9 = International Classification of Disease, ninth revision

aOR = adjusted odds ratio

**Competing interests**

The authors have no competing interests.

**Authors' contributions**

Bing-Fang Hwang is responsible for study concept and design, integrity of the data, the accuracy of the data analysis, and drafting of the manuscript; Jouni JK Jaakkola for critical revision of the manuscript for important intellectual content; How-Ran Guo for study concept and design, and study supervision. All authors read and approved the final manuscript.

**Acknowledgements**

This study was partially supported by grant #NSC 92-2320-B-434-001 from National Science Council. We thank the Taiwan Water Supply Corporation (TWSC) and Taipei Water Department providing water monitoring data and the Department of Health for access to the birth registration.

## References

1. Rook J. **Formation of haloforms during chlorination of natural waters.** *Water Treatment Exam.* 1971; **23**:90-92.
2. Tibbets J. **What's in the water: the disinfection dilemma?** *Environ Health Perspect.* 1995; **103**:30-34.
3. Nieuwenhuijsen M, Toledano M, Eaton N, et al. **Chlorination disinfection by-products in water and their association with adverse reproductive outcomes: a review.** *Occup Environ Med.* 2000; **57**:73-85.
4. Aschengrau A, Zierler S, Cohen A. **Quality of community drinking water and the occurrence of late adverse pregnancy outcomes.** *Arch Environ Health.* 1993; **48**:105-113.
5. Bove F, Fulcomer M, Klotz J, et al. **Public drinking water contamination and birth outcome.** *Am J Epidemiol.* 1995; **141**:850-862.
6. Dodds L, King W, Woolcott C, et al. **Trihalomethanes in public water supplies and adverse birth outcomes.** *Epidemiology.* 1999; **10**:233-237.
7. Klotz JB, Pynch LA. **Neural tube defects and drinking water disinfection by-products.** *Epidemiology.* 1999; **10**:383-390.
8. Magnus P, Jaakkola JJK, Skrondal A, et al. **Water chlorination and birth defects - a nationwide registry based study.** *Epidemiology.* 1999; **10**:513-517.
9. Hwang BF, Jaakkola JJK. **Water chlorination and birth defects: A systematic review and meta-analysis.** *Arch Environ Health.* 2003; **58**:83-91.
10. Cedergren MI, Selbing AJ, Lofman O, et al. **Chlorination byproducts and nitrate in drinking water and risk for congenital cardiac defects.** *Environ Res.* 2002; **89**:124-130.

11. Shaw GM, Ranatunga D, Ouach T, et al. **Trihalomethane exposures from municipal water supplies and selected congenital malformations.** *Epidemiology*. 2003; **14**:191-199.
12. Hwang BF, Magnus P, Jaakkola JJK. **Risk of specific birth defects in the relation to chlorination and among of the natural organic matter in the water supply.** *Am J Epidemiol*. 2002; **156**:374-382.
13. World Medical Assembly. **Declaration of Helsinki: Recommendations guiding physicians in biomedical research involving human subjects.** *Bull Pan Am Health Organ* 1990; **24**:606-09.
14. Petitti DB. **Meta-analysis, decision analysis, and cost-effectiveness analysis.** Methods for quantitative synthesis in medicine. *Oxford University Press*, 1994; 256pp.
15. DerSimonian R, Laird N. **Meta-analysis in clinical trials.** *Control Clin Trials* 1986; **7**:177-188.
16. Lin CM, Lee PC, Teng SW, et al. **Validation of the Taiwan birth registration using obstetric records.** *J Formos Med Assoc* 2004; **103**:297-301.
17. Klotz JB, Pyrch LA. **A case-control study of neural tube defects and drinking water contaminants, NTIS.** *U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA* 1998.(NTISPB98-111644).
18. Dodds L, King W. **Relation between trihalomethane compounds and birth defects.** *Occup Environ Med*. 2001; **58**:443-446.
19. Ritz B, Fruin S, Chapa G, et al. **Ambient air pollution and risk of birth defects in southern California.** *Am J Epidemiol*. 2002; **155**:17-25.

20. Totedano MB, Nieuwenhuijsen MJ, Best N, et al. **Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England.** *Environ Health Perspect* 2005; **113**:225-232.
21. Brown-Woodman PD, Hayes LC, Huq F, et al. **In vitro assessment of the effect of halogenated hydrocarbons: Chloroform, dichloromethane, and dibromoethane on embryonic development of the rat.** *Teratology*. 1998; **57**:321-333.
22. Chen J, Thirkill TL, Lohstroh PN, et al. **Bromodichloromethane inhibits human placental trophoblast differentiation.** *Toxicol Sci*. 2004; **78**:166-174.
23. Mayeno AN, Yang RSH, Reisfeld B. **Biochemical reaction network modeling: Predicting metabolism of organic chemical mixtures.** *Environ Sci Technol*. 2005; **39**: 5363-5371.

**TABLE 1. Characteristics of the study population (N = 396,049) according to the categories of exposure, Taiwan, 2001-2003.**

Characteristic	TTHMs (0-4 µg/L) <b>Reference</b> N (%)	TTHMs (5-9 µg/L) <b>Low</b> N (%)	TTHMs (10-19 µg/L) <b>Medium</b> N (%)	TTHMs (20+ µg/L) <b>High</b> N (%)	Total N (%)
Total	181,985 (100%)	55,950 (100%)	82,797 (100%)	75,317 (100%)	396,049 (100%)
Gender of infant	$\chi^2$ (df=3)=1.164 p-value=0.762				
Male	95,027 (52.2%)	29,128 (52.1%)	43,155 (52.1%)	39,413 (52.3%)	206,723(52.2%)
Female	86,958 (47.8%)	26,822 (47.9%)	39,642 (47.9%)	35,904 (47.7%)	189,326 (47.8%)
Maternal age	$\chi^2$ (df=6)=1466 p-value=0.001				
<20 years	6,419 (3.5%)	1,838 (3.3%)	3,499 (4.2%)	3,461 (4.6%)	15,217 (3.8%)
20-34	156,087 (85.8%)	46,871 (83.8%)	72,402 (87.4%)	65,947 (87.6%)	341,307 (86.2%)
35-	19,476 (10.7%)	7,241 (12.9%)	6,893 (8.3%)	5,909 (7.8%)	39,519 (10.0%)
Maternal high fever ( body temp of pregnant women after deliver >38 °C)	$\chi^2$ (df=3)=0.0334 p-value=0.998				
Yes	394 (0.2%)	159 (0.3%)	170 (0.2%)	138 (0.2%)	861 (0.2%)
No	181,591 (99.8%)	55,791 (97.7%)	82,627 (99.8%)	75,179 (99.8%)	395,188 (99.8%)
Maternal diabetes mellitus	$\chi^2$ (df=3)=0.0335 p-value=0.998				
Yes	418 (0.2%)	189 (0.3%)	179 (0.2%)	144 (0.2%)	930 (0.2%)
No	181,567 (99.8%)	55,761 (99.7%)	82,618 (99.8%)	75,173 (99.8%)	395,119 (99.8%)
Plurality	$\chi^2$ (df=3)=48.6 p-value=0.001				
Singleton	176,791 (97.1%)	54,383 (97.2%)	80,755 (97.5%)	73,442 (97.5%)	385,371 (97.3%)
Multiple birth	5,194 (2.9%)	1,567 (2.8 %)	2,042 (2.5%)	1,875 (2.5%)	10,678 (2.7%)
Population density (no of people/km <sup>2</sup> )*	$\chi^2$ (df=6)=23405 p-value=0.001				
<1000	29,881 (16.4%)	6,697 (12.0%)	20,387 (24.6%)	26,392 (35.2%)	83,357 (21.1%)
1000-5000	71,005 (39.0%)	27,257 (48.7%)	23,569 (28.5%)	31,823 (42.5%)	153,654 (38.8%)
>5000	81,099 (44.6%)	21,996 (39.3%)	38,841 (46.9%)	16,738 (22.3%)	158,674 (40.1%)

**TABLE 2.** Characteristics of included population, excluded population and total population in Taiwan 2001-2003.

Characteristics	Included population		Excluded population		Total population	
	N	%	N	%	N	%
Total	396,049	100	325,240	100	721,289	100
Gender of infant	$\chi^2$ (df=1)=0.0008 p-value=0.9774					
Male	206,723	52.2	170,385	52.4	377,108	52.3
Female	189,326	47.8	154,524	47.6	343,850	47.7
Maternal age	$\chi^2$ (df=2)=0.3437 p-value=0.8421					
<20 years	15,217	3.8	16,223	5.0	31,440	4.4
20-34	341,307	86.2	282,381	86.8	623,688	86.5
35-	39,519	10.0	26,631	8.2	66,150	9.2
Maternal high fever ( body temp of pregnant women after deliver >38 °C)	$\chi^2$ (df=1)=0.0002 p-value=0.9997					
Yes	861	0.2	583	0.2	1,444	0.2
No	395,188	99.8	324,657	99.8	719,845	99.8
Maternal diabetes mellitus	$\chi^2$ (df=1)=0.0201 p-value=0.8874					
Yes	930	0.2	876	0.3	1,806	0.3
No	395,119	99.8	324,364	99.7	719,483	99.7
Plurality	$\chi^2$ (df=1)=0.0019 p-value=0.9649					
Singleton	385,371	97.3	316,903	97.4	702,274	97.4
Multiple birth	10,678	2.7	8,337	2.6	19,015	2.6
Population density (no of people/km <sup>2</sup> )*	$\chi^2$ (df=2)=5.0533 p-value=0.0799					
<1000	83,357	21.1	94,304	29.5	177,661	24.8
1000-5000	153,654	38.8	144,069	45.0	297,723	41.6
>5000	158,674	40.1	81,817	25.6	240,491	33.6

**Table 3.** Prevalences and prevalence odds ratios of the most common birth defects according to exposure to trihalomethanes in Taiwan 2001-2003.

Outcomes		TTHM 0-4 $\mu$ g/L <b>Reference</b>	TTHM 5-9 $\mu$ g/L <b>Low</b>	TTHM 10-19 $\mu$ g/L <b>Medium</b>	TTHM 20+ $\mu$ g/L <b>High</b>
<b><i>Any birth defect</i></b>					
N	2,148	978	368	421	381
P (%)	0.540	0.54	0.660	0.510	0.510
OR		1.00	1.23	0.95	0.94
aOR		1.00	1.21	0.97	1.00
(95% CI)			(1.07-1.36)	(0.86-1.08)	(0.89-1.13)
<b>Anencephalus</b>					
N	43	19	9	2	13
P (%)	0.011	0.010	0.016	0.002	0.017
OR		1.00	1.54	0.23	1.65
aOR		1.00	1.59	0.23	1.96
(95% CI)			(0.72-3.52)	(0.05-1.01)	(0.94-4.07)
<b>Hydrocephalus</b>					
N	118	58	24	19	17
P (%)	0.030	0.032	0.043	0.023	0.023
OR		1.00	1.35	0.72	0.71
aOR		1.00	1.36	0.71	0.74
(95% CI)			(0.85-2.20)	(0.42-1.20)	(0.43-1.28)
<b>Ventricular septal defects</b>					
N	59	27	6	8	18
P (%)	0.015	0.015	0.011	0.010	0.024
OR		1.00	0.72	0.65	1.61
aOR		1.00	0.74	0.65	1.81
(95% CI)			(0.31-1.80)	(0.29-1.43)	(0.98-3.35)
<b>Atrial septal defect</b>					
N	19	8	5	2	4
P (%)	0.005	0.004	0.009	0.002	0.005
OR		1.00	2.03	0.55	1.21
aOR		1.00	2.15	0.53	1.33
(95% CI)			(0.70-6.60)	(0.11-2.49)	(0.39-4.58)
<b>Tetralogy of Fallot</b>					
N	24	13	6	3	2
P (%)	0.006	0.007	0.011	0.004	0.003
OR		1.00	1.50	0.51	0.37
aOR		1.00	1.60	0.46	0.32
(95% CI)			(0.61-4.23)	(0.13-1.61)	(0.07-1.47)
<b>Cleft palate</b>					
n	358	155	55	84	64
P (%)	0.090	0.085	0.098	0.101	0.085
OR		1.00	1.15	1.19	1.00
aOR		1.00	1.15	1.20	0.98
(95% CI)			(0.84-1.56)	(0.91-1.55)	(0.73-1.32)

Cleft lip					
n	129	52	15	28	34
P (%)	0.033	0.029	0.027	0.034	0.045
OR		1.00	0.94	1.18	1.58
aOR		1.00	0.94	1.17	1.56
(95% CI)			(0.53-1.68)	(0.74-1.86)	(1.00-2.41)
Renal agenesis & dysgenesis					
n	76	33	14	13	16
P (%)	0.019	0.018	0.025	0.016	0.021
OR		1.00	1.38	0.87	1.17
aOR		1.00	1.33	0.92	1.27
(95% CI)			(0.71-2.48)	(0.48-1.75)	(0.69-2.33)
Hypospadias					
n	72	43	8	14	7
P (%)	0.018	0.024	0.014	0.017	0.009
OR		1.00	0.61	0.72	0.39
aOR		1.00	0.59	0.76	0.47
(95% CI)			(0.28-1.26)	(0.41-1.38)	(0.21-1.04)

\*cOR: crude odds ratio; aOR: adjusted odds ratio. Logistic regression analysis adjusting for maternal age, plurality, and population density. \*Estimates not given for the following rare outcomes: spinal bifida (n=14) microcephalus (n=9); endocardial cushion defects (n=7); transposition of great vessels (n=12); choanal atresia (n=2); dysplasia of lung (n=9); congenital cystic kidney (n=24).

**Table 4. Summary of the results from studies in Sweden, Norway, California, and Taiwan focusing on six most common specific birth defect.**

	Sweden 2000	Norway 2002	California 2003 <sup>(1)</sup>	California 2003 <sup>(2)</sup>	Taiwan 2007
Type of study	Cross-sectional, population-based	Cross-sectional, population-based	Case-control	Case-control	Cross-sectional, population-based
Study population	115,801 births	184,676 births	539 controls	481 controls	396,409 births
No of birth Defects	1,842	5,764	538	265	2,148
Exposure	Chlorine dioxide	Chlorination, high color	TTHMs 50-74 µg/l	TTHMs 50-74 µg/l	TTHMs >=20 µg/l
Reference	None	No chlorination, low color	TTHMs 0 µg/l	TTHMs 0 µg/l	TTHMs 0-4 µg/l
Anencephalus		0.79 (0.15-4.23) (n = 46)	0.55 (0.26-1.10) (n=164)	1.80 (0.76-4.40) (n=89)	1.96 (0.94-4.07) (n=43)
Hydrocephalus	1.50 (0.30-7.30) (n = 36)	2.70 (0.77-9.51) (n = 68)			0.74 (0.43-1.28) (n = 118)
Ventricular septal defect		1.81 (1.05-3.09) (n = 279)			1.81 (0.98-3.35) (n = 59)
Atrial septal defect		0.92 (0.24-1.50) (n = 73)			1.33 (0.39-4.58) (n = 19)
Cleft palate		1.07 (0.35-3.27) (n = 95)		1.00 (0.32-3.40) (n=58)	0.98 (0.73-1.32) (n = 358)
Cleft lip		2.01 (0.63-6.46) (n = 67)		1.90 (0.81-4.50) (n=117)	1.56 (1.00-2.41) (n = 129)

\*California 2003<sup>(1)</sup>: Shaw et. al. Study 1; California 2003<sup>(2)</sup>: Shaw et. al. Study 2

**Table 5.** A comparison of the results from the present, Sweden, Norway, and California studies. Summary odds ratios for the relations between exposure to disinfection by-products in the exposure vs. reference category.

Birth defect	Study area	Fixed-Effects	Random-Effects	Q-statistic/ P-value*
		Model Summary OR (95 % CI)	Model Summary OR (95% CI)	
Anencephalus	Norway 2002	1.13 (0.74-1.73)	1.15 (0.56-2.36)	7.55/0.056
	California 2003 <sup>(1)</sup>			
	California 2003 <sup>(2)</sup>			
	Taiwan 2007			
	Norway 2002	0.85 (0.51-1.43)	0.91 (0.40-2.51)	
	California 2003 <sup>(1)</sup>			
	California 2003 <sup>(2)</sup>			
	Norway 2002	0.98 (0.61-1.59)	0.98 (0.38-2.51)	
	California 2003 <sup>(1)</sup>			
	Taiwan 2007			
	Norway 2002	1.73 (1.01-2.96)	1.73 (1.01-2.96)	
	California 2003 <sup>(2)</sup>			
Hydrocephalus	Sweden 2000	0.95 (0.59-1.50)	1.21 (0.52-2.81)	3.76/0.152
	Norway 2002			
	Taiwan 2007			
Ventricular septal defects	Norway 2002	1.81 (1.21-2.71)	1.81 (1.21-2.71)	0.0001/0.99
	Taiwan 2007			
Atrial septal defect	Norway 2002	0.97 (0.61-1.52)	0.97 (0.61-1.52)	0.30/0.59
	Taiwan 2007			
Cleft palate	Norway 2002	0.99 (0.75-1.31)	0.99 (0.74-1.31)	0.02/0.99
	California 2003 <sup>(2)</sup>			
	Taiwan 2007			
Cleft lip	Norway 2002	1.66 (1.15-2.40)	1.66 (1.14-2.42)	0.28/0.87
	California 2003 <sup>(2)</sup>			
	Taiwan 2007			

\*P<0.05 indicates that a random-effects model is more appropriate.

\*California 2003<sup>(1)</sup>: Shaw et. al. Study 1; California 2003<sup>(2)</sup>: Shaw et. al. Study 2