

Author's response to reviews

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

Authors:

Bing-Fang Hwang (bfhwang@mail.cmu.edu.tw)
Jouni JK Jaakkola (j.jaakkola@bham.ac.uk)
How-Ran Guo (hrguo@mail.ncku.edu.tw)

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Author's response to reviews: see over

A Point-by-Point Response**EH/2007/MS: 1176667096156881****Water Disinfection By-products and the Risk of Specific Birth Defects: A Population-based Cross-Sectional Study in Taiwan.****Reviewer 1 (Mika Gissler) Comments for the Author.****What next?: Accept without revision****Level of interest: An article whose findings are important to those with closely related research interests****Quality of written English: Acceptable****Statistical review: No, the manuscript does not need to be seen by a statistician.****Reviewer 2 (sylvaine Cordier) Comments for the Author.****General****This paper relies on birth certificates corresponding to a subsample of Taiwanese births for the ascertainment of associations between a selected number of birth defect types and exposure to disinfection by-products.****Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)**

- 1. There are many approximations or unsupported assertions and this manuscript appears somewhat unfinished. Several times, justifications are given several pages after the reader expects to find them.**

This has been improved in the revised version.

- 2. Study population: Almost half of the births occurring in Taiwan between 2001 and 2003 have been excluded for “insufficient information on disinfection practice in the municipality”. It appears from the following sentence and later in Table 2, that these births were probably excluded because the region where they occurred was served by more than “one type of waterworks”. What does “one type of waterworks” mean is unclear: does it refer to networks? To water treatment?**

We focused on municipalities where all waterworks either used chlorination or did not use chlorination to eliminate ecologic bias. The waterworks refer to water treatment.

- 3. The five water regions selected appear to include roughly half of the Taiwanese births and characteristics of births included and excluded are presented in Table 2, but some general description of the area under study compared to the whole country is necessary already at this stage.**

In table 4, we compared the populations included and excluded from the analyses, and the total population (page 23). There were no statistically significant differences (χ^2 test; $p > 0.05$) between the characteristics of the included population and the total population.

- 4. Health outcomes: What is the reliability of birth defect reporting on birth certificates in Taiwan? A detailed answer is given in the discussion, but precisions should be given in this section on the reporting process (actors, timing, validation ...).**

According to the law, all live births in Taiwan must be reported within 15 days after birth by parents to the Taiwan Local Household Registry, which is managed by both the Department of Interior Affairs and the Department of Health. In addition, Taiwanese pregnant women are almost all covered by national 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. The validation of the Taiwanese birth registration 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 k statistics was 0.92) (Lin et al. 2004). This has been revised in the health outcomes (page 5 and 6).

- 5. The strategy followed for the choice of the birth defects under study is not clearly justified. The decision made by the authors to study single defects rather one type of "organ groups" is debatable especially when considering the small numbers involved (Table 3). Chromosomal anomalies are not mentioned. Depending on the importance of prenatal diagnosis in Taiwan, the pertinence of studying anencephalus at birth must be discussed. Among neural tube defects, spina bifida is much more frequent than anencephalus but not studied.**

Both the etiologic factors and mechanisms of single defects are known or suspected to vary within a group of defects which are mainly defined by anatomical site. This means that focusing on a single defect will eliminate the influence ("noise") of heterogeneity in causes and mechanisms and allows estimation of the true relation of interest. This will improve the validity of the effect estimates, although the precision

will be compromised due to smaller number of cases. Our strategy is based on the logic that validity is prior to precision.

We studied neural tube defects including, anencephalus (n=43), spina bifida (n=14), microcephalus (n=9), and hydrocephalus (n=118). Estimates are not given for the rare outcomes: spinal bifida (n=14), microcephalus (n=9) in the table 3 footnote. Among neural tube defects, we did not identified spina bifida is much more prevalent than anencephalus. It's that possible that 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. Chromosomal anomalies have been added in the table 5 (page 25). The effect estimate for chromosome anomalies was slight elevated in low (1.25, 0.95-1.65), but not in medium (0.93, 0.70-1.24) and high exposure categories (0.90, 0.66-1.24), as compared with the reference category.

6. In the background statements, the authors point at obstructive urinary tract defects as candidate health effects as assessed in the Norwegian study they plan to reproduce, but they choose not to study them.

The outcome of obstructive urinary tract has been added in the results and table 5 (page 25). The effect estimates for obstructive urinary tract defect (1.44, 0.66-3.14) were slightly elevated in the high exposure category compared with reference category. This result is consistent with Norwegian study (1.99, 0.66-5.96) in the same exposure contrast.

7. Reliability of reporting of hypospadias has been discussed by specialists of birth defect registries and the study of this birth defect based on birth records may not be valid.

We agree hypospadias may not be valid based on birth defect registration. Nevertheless we think the effect estimates are of interest to the reader, although they have to use their own judgment of the validity.

8. A list of birth defects “excluded” is given (bottom of page 5): the reason is not clear since the list of included defects is already defined. Are multiple anomalies included?

A list of birth defects “excluded” has been removed. We did not include multiple anomalies.

- 9. Exposure assessment: Authors mention using mother's place of residence during pregnancy: is this information available on birth certificate? Place of residence at birth is not always the relevant address (beginning of pregnancy). What is the likely importance of this problem (changes in residence during pregnancy) in Taiwan? this should be discussed.**

There place of residence at birth is available on birth certificate. We agree that that the place of residence at birth may not always the relevant address. Unfortunately, we do not have information on women who moved during pregnancy. We discussed this issue in the discussion part (page 12-13).

- 10. It is not clear how individual TTHM levels were estimated and on which period (date of conception? whole pregnancy?).**

We assessed exposure by calculating a weighted average of the modeled quarterly TTHM estimates for the appropriate waterworks during the date of conception and the date of birth. The weighting was based on the proportion of the trimester falling into each quarterly period.

- 11. Covariates: What is the rationale for including "high fever" after delivery among the covariates since the risk period for the birth defects studied is early pregnancy ?**

We try to compare the characteristics of included population, excluded population and total population and show there were no statistically significant differences (χ^2 test; $p > 0.05$) between the characteristics of the included population and the total population. We did not adjust for high fever in the multiple logistic regression analysis (Table 5).

- 12. Statistical methods: Justifications must be given for the choice of the reference category for TTHM levels (0-4 #g/l) and of exposure categories. The number of measurements available, their distribution, range etc...must be described. All these details should be added to the Exposure Assessment section.**

Under the regulations operating during the study period, the standard sampling frequency for TTHMs was a minimum of four samples per year for each waterwork. The number of measurements and distribution of TTHMs between three exposure categories and reference category were shown in Table 1 and added in the exposure assessment section (page 6).

13. The last sentence of page 7 is not clear: how will a stratified analysis “evaluate the potential role of residual confounding”?

This sentence has been corrected as “ To evaluate the effect modification, we” (page 7). The detail stratified analysis was added in the following table 2. Due to small sample size, we only present the defect of cleft palate. The results did not show any effect modification.

14. No method is described (or used) for the study of dose-response relationship. The strategy for the meta-analysis is described at this point as including the present study and “the other available Norwegian study”. In fact several other studies were included in the meta-analysis presented in Table 5...

Chi-square test for trend in binominal proportions was performed to determine whether or not the dose-response relation exists (page 7). However, only the effect estimate for cleft palate show a clear dose-response relationship ($p=0.03$).

For meta analysis, we have substantially revised the text on page 8.

15. Results: No test for trend is presented in the study of the association between TTHMs levels and prevalence of birth defects.

Chi-square test for trend in binominal proportions was performed to determine whether or not the dose-response relation exists. However, only a clear association between the levels of TTHMs and the prevalence of cleft palate ($p=0.03$) was found in the present study (page 9).

16. Exclusively estimates relative to the high exposure category are underlined, even in the presence of high imprecision (renal agenesis & dysgenesis). Several inconsistent findings (many estimates in the low category of exposure are elevated, including a significantly increased risk for “any birth defect”) or inverse associations (hypospadias) are not underlined. Cleft palate cases are much more prevalent than cleft lip which is unusual, even among Asian populations

We have modified the text and the Tables 5, 6, and 7 according to the reviewer’s comment. The cleft lip with/without palate cases are much more prevalent than cleft palate. We also revised the text in the results part (page 9, 10).

- 17. For some categories of defects (atrial septal defects, tetralogy of Fallot), numbers are very small and analyses using four categories of exposure are not very informative (Table 3).**

We also try to combine four categories to contrast low (<10 ug/l) and high (> 10 ug/l) exposure. The effect estimates show similar trend for atrial septal defects (adjusted OR 0.70, 95% CI 0.26-1.87) and tetralogy of Fallot (0.35, 0.13-1.05).

- 18. Whereas at least nine previous studies have been referenced in the background information, only four are presented in Table 4 (one only was announced in the Statistical analysis paragraph).**

Although at least nine previous studies have been assessed the water disinfection by products and birth defects, only 5 of these studies focussed on the specific birth defects (Table 6, page 26). This statistical analysis paragraph has been revised (page 8).

- 19. Summary estimates (Table 5) and their interpretation are highly conditioned by the choice of studies included in the meta-analysis (see above) and the exclusion of others. This point should be carefully described and justified in the methods section.**

We searched the Medline data retrieval system years 1966-2007. Three authors independently reviewed the articles, extracted data, and assessed the validity of the studies. There were only 5 studies that focussed on the seven most common specific birth defects in the relation to water disinfection. This has been added in the methods and results sections (page 8, 9, 10).

- 20. Only one reference is given for two studies by Shaw mentioned in California.**

Although Shaw conducted two different studies in California, this work was published by one paper. Therefore, there is only one reference.

- 21. Discussion: In several instances, the potential misclassification errors in birth registration are discussed (page 11; line 8 and 23) and presented as “likely to be random”. However on page 12, lines 10-11, adjustment for population density is presented as a way to “eliminate partly underreporting bias between regions”. As can be seen in Table 1, regions with high TTHM levels appear to be more rural. I suggest to substantiate the issue of potential confounding by population density and to present**

somewhere (Table 2?) the prevalence of birth defects according to population density.

This sentence has been revised (page 12). Adjustment for population density adjusted indirectly for municipal differences in these behavior factors, but also eliminated partly regions differences with TTHM levels between rural and urban.

22. The discrepancy between the number of studies discussed in the section “Synthesis with previous knowledge” page 13, and the number of studies included in the pooled analysis should be clearly explained. For example, why four available studies when discussing neural tube defects (line 8) whereas eight studies are mentioned? This probably relates to the choice to study only individual defects instead of organ groups. I therefore suggest that the authors perform the meta-analysis of broader organ groups including the corresponding studies, in addition to the individual defects they have chosen. This is needed for a proper assessment of the associations found.

We identified and synthesized the epidemiological evidence for adverse effects of prenatal exposure to chlorination by-products on birth defects. We have previously presented results from a meta-analysis focusing on five major groups of birth defects. This work has been published in the Archive of Environmental Health 2003 (Hwang et al. 2003).

In the present study, we focused on specific birth defects. Only the five previous studies have previously explored systematically the main specific birth defects and therefore a meta-analysis of the results from five similar studies was appealing.

23. In Europe, urinary tract defects such as obstructive defects, are not considered as rare. What is the prevalence in Taiwan (page 14, line 6)?

The prevalence of obstructive urinary tract defects in Taiwan is 0.12% (Table 5, page 25).

24. The discussion on biological mechanisms includes only THMs whereas they should only be considered as markers of the whole mixture of disinfection by-products, also when discussing hazards. This discussion also includes a presentation of a genetic mechanism alternative to teratogenicity, affecting maternal gametes. If the authors consider this hypothesis as a serious alternative, they should substantiate it and: 1) study genetically determined birth defects; 2) assess preconceptional exposures.

Although THMs are the most prevalent in chlorinated water, they may only be considered as markers of whole mixture of disinfection by-products. Some animal studies also show reproductive and developmental toxicity of haloacetic acid, non-volatile disinfection by-products, such as Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) (Nieuwenhuijsen et al. 2000). Further detailed toxicological assessments of mixtures of chlorination by-products are also needed, as humans are most commonly exposed to complex mixtures of these compounds rather than to a single compound (Brooker 2000).

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

This has been corrected.

25. Take out all Chi Square values from tables 3-4.

We have taken all Chi Square values out from Table 3-4 in the revised version.

26. Include in Table 6 reference numbers for the studies selected

This has been added in the revised version (page 26).

27. Table 7 should indicate more clearly which studies are included in the summary estimates.

We have added reference number in the Table 7 (page 28 footnote).

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Reviewer 3 (Manolis Kogevinas) Comments for the Author.**General**

The authors report of a large study in Taiwan associating 9 congenital malformations with levels of THMs in drinking water. The issue evaluated is important and has been discussed for quite some time. Exposure classification was based on routine measurements. An association is found between the highest exposure levels and specific malformations although no effect (actually a protective effect) was found for hypospadias. A meta analysis was conducted combining results from other similar studies. An important aspect of this study is the large sample size. Similar to many studies based on routinely collected data, this study has limited information on exposures and potential confounders. The authors could probably have provided a more balanced summary of the findings. Finally, the text could be clearer when discussing the meta analysis.

We have added “a negative association was found for hypospadias” in the results section (page 9) and provided more balance summary of finding in the abstract. We also discuss the meta analysis issues in the discussion section (page 13).

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

- 1. The authors, mainly in the abstract, are reporting the most extreme results. It is true that they did have some significant increases in risk comparing the highest exposed group with the lowest for anencephalus, ventricular septal defects and cleft lip, but their is no dose response in the ORs by increasing THM level. The authors should be somehow more careful in interpreting these findings and should provide a . They should also comment more adequately the decreased risk for hypospadias.**

A negative association was found for hypospadias, which is probably explained by less valid of diagnosis based on birth registration and lead to misclassification of disease. Therefore the effect estimate would be bias toward to the null or away form the null (page 15).

- 2. The authors should discuss the range of exposures examined. The difference between exposure groups is rather narrow and, given exposure misclassification, it would be expected not to find large effects even if they existed. It would be useful if the authors provided more detailed information on the distribution of exposure levels in the highest category.**

The distribution of exposure levels in three categories was shown in the Table 2 and added in the exposure assessment section (page 6, 21). The range of the level of TTHMs was from 0.85 µg/L through 32.65µg/L. Although the exposure categories are narrow, the risk of ventricular septal defects was associated with the level of TTHMs, consistent with English and Welsh and Norwegian studies.

- 3. In the discussion the authors could comment how do their exposure levels compare with those of other studies.**

The present and three previous studies using routine measurements of trihalomethanes (Shaw et al. 2003; Nieuwenhuijsen et al. 2007) constituted a rather homogeneous group, but different exposure contrasting. Also a Sweden study used water source and chlorination practice as the basis for exposure assessment (Cedergren et al. 2002), and 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 (Hwang et al. 2002). We added in the discussion section (page 13).

- 4. It is not clear what exactly have the authors done with the meta analysis. This has to be written in a much clearer way. In some parts it appears as if they compared only with the Norwegian cohort while in the tables they quote other studies as well.**

We did all combination of present study and all available studies with meta analysis in the Table 7 according to study area (page 27, 28). We have written much more clear in the revised version (page 8, 9, 10, 13).

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Table 1. the numbers of measurement, and distribution of TTHMs between three exposure categories and reference category.

Exposure categories	No of samples	Mean±SD (µg/L)	Minimum (µg/L)	Maximum (µg/L)	Median (µg/L)
Reference (TTHMs 0-4 µg/L)	528	3.64±0.95	.85	4.96	3.80
Low (TTHMs 5-9 µg/L)	168	5.57±0.96	5.00	9.50	5.00
Medium (TTHMs 10-19 µg/L)	240	16.48±2.94	10.68	19.53	15.57
High (TTHMs 20+ µg/L)	228	23.24±2.27	20.35	32.65	22.70

Table 2 Stratify analysis for cleft palate by covariates

Maternal age	<20 years	20-34 years	>=35 years
No. of cleft palate	N=8	N=108	N=13
Exposure categories	cOR (95%)	cOR (95%)	cOR (95%)
Reference	1	1	1
Low	-	0.91 (0.48-1.72)	1.34 (0.34-5.38)
Medium	1.84 (0.26-13.0)	1.13 (0.68-1.87)	1.41 (0.35-5.65)
High	3.71 (0.68-20.3)	1.56 (0.98-2.49)	0.55 (0.07-4.56)
Plurality	Single	Multiple births	
No. of cleft palate	N=126	N=3	
Exposure categories	cOR (95%)	cOR (95%)	
Reference	1	1	
Low	0.96 (0.54-1.70)	-	
Medium	1.12 (0.70-1.79)	5.09 (0.46-56.2)	
High	1.61 (1.04-2.48)	-	
Population density (no of people/km²)	<1000	1000-5000	>5000
No. of cleft palate	N=32	N=49	N=48
Exposure categories	cOR (95%)	cOR (95%)	cOR (95%)
Reference	1	1	1
Low	0.89 (0.20-4.07)	1.79 (0.83-3.86)	0.28 (0.07-1.20)
Medium	1.03 (0.39-2.70)	2.07 (0.96-4.47)	0.80 (0.39-1.67)
High	1.47 (0.65-3.36)	1.53 (0.71-3.31)	1.86 (0.90-3.87)

References:

Brooker SM. **NTP taps disinfection by-products for study.** *Environ Health Perspect* 2000; **108**:pA64-67.

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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.

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Nieuwenhuijsen MJ, Toledano MB, Bennett J, et al. **Chlorination disinfection by-products and risk of congenital anomalies in England and Wales.** *Environ Health Perspect* 2007 online 6 November.

Shaw GM, Ranatunga D, Ouach T, et al. **Trihalomethane exposures from municipal water supplies and selected congenital malformations.** *Epidemiology.* 2003; **14**:191-199.