Re-Analysis of Data from Two Chloroform Epidemiological Studies: Wennborg et al. (2000) and Infante-Rivard (2004)

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On November 4, 2004 the Developmental and Reproductive Toxicant (DART) Identification Committee, the State's qualified experts for reproductive toxicity for Proposition 65, met to consider whether chloroform had been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity. The committee voted not to list this chemical as known to cause reproductive toxicity under Proposition 65 for the either developmental, male reproductive or female reproductive toxicity endpoints. However, the Committee did request that the Office of Environmental Health Hazard Assessment (OEHHA) try to obtain additional information regarding re-analyses of findings from two epidemiologic studies, one by Wennborg et al. (2000), and the other by Infante-Rivard (2004). OEHHA contacted the primary authors of these articles and, after discussion of the issues raised by the DART Committee, the authors have provided OEHHA with the results of the requested re-analyses. Below is a description of the specific requests made of the authors and the results from their re-analyses.

Re-analysis from Dr. Wennborg:

As summarized in the draft Hazard Identification Document on Chloroform (OEHHA, 2004: pages 13-14), Dr. Wennborg and coauthors conducted an occupational study of women, which examined exposure to chloroform in association with pregnancy outcomes. The study reported a weak association between women working with chloroform during the time before conception and the occurrence of spontaneous abortion (SAB) (odds ratio = 2.3; 95% confidence interval 0.9 – 5.9). The regression analysis resulting in this finding included adjustment for mother's age and previous SAB. However, as discussed at the DART Committee meeting, it was not clear from the study whether the previous SABs occurred before or during the time when the women were exposed to chloroform. If the women were exposed to chloroform and/or other chemicals at the time the previous SAB occurred, including this variable in the regression analysis could have resulted in over control, which would have biased the results. Therefore, following the direction of the DART Committee, OEHHA requested that Dr. Wennborg either: 1) verify that the SABs occurred before exposure to the chloroform, or 2) rerun the statistical analyses of the data omitting the previous SABs.

Dr. Wennborg responded that previous SABs included SABs that were "previous" in relation to the pregnancy in question. Thus these did include SABs that occurred while the women were occupationally exposed to chemicals. Therefore, she reran the analysis excluding the previous SABs, and reported the following results. The odds ratio was 2.1, with 95% confidence interval 1.1 – 4.0. Thus the odds ratio was about the same (2.1 vs.

2.3), but the 95% confidence interval was smaller (1.1 – 4.0 vs. 0.9 – 5.9), and now statistically significant. Dr. Wennborg noted that the analysis in 2000 was performed with STATA 6.0, and the new analysis with STATA 8.0. STATA is a statistical data analysis program similar to programs such as SAS.

Re-analysis from Dr. Infante-Rivard:

As summarized in the draft Hazard Identification Document on Chloroform (OEHHA, 2004: pages 20-22), Dr. Infante-Rivard conducted a case-control study that examined the association between exposure to chloroform and fetal growth. The study also tested for gene-environment interactions to determine whether effects of chloroform exposure were modified by newborn and genetic variants. In analyzing the effect of exposure to trihalomethanes (THMs) and chloroform, Dr. Infante-Rivard used the 90^{th} percentile as a cutoff, thus considering the top 10^{th} percentile of individuals as exposed. The author concluded that the findings suggest exposure to THMs at the highest levels can affect fetal growth but only in genetically susceptible newborns. The results are not statistically significant for chloroform. However, as discussed at the DART committee meeting, the size of the sample of women in the exposed group was small when the 90^{th} percentile cutoff was used. This may have limited the power of the study to detect an effect, if one were present. Therefore, following the direction of the DART committee, OEHHA requested that Dr. Infante-Rivard reanalyze the data using a less conservative cutoff. Table 1 below shows the results of the analysis conducted using the 90^{th} percentile cutoff, as reported in the study, as well as the reanalysis using the 75^{th} percentile cutoff. These results using the 75^{th} percentile were not statistically significant for either THMs or chloroform.

Dr. Infante-Rivard pointed out that she disagreed with choosing a 75^{th} percentile cutoff since she believed one should choose the cutoff based on where effects are likely. The levels of chloroform exposure in this study were considerably lower, even at the 90^{th} percentile, than those in studies that had reported a statistically significant effect.

Table 1. Adjusted ORs (95% CIs) for exposure to THMs (chloroform and total THMs) in drinking water measured as average level at the tap, according to newborn and maternal polymorphisms in the CYP2E1 and MTHFR genes.

Gene	OR (95% CI) Using a 90th percentile cutoff		OR (95% CI) Using a 75th percentile cutoff	
	Chloroform	Total THMs	Chloroform	Total THMs
Newborns				
*CYP2E1*5* (G1259C)				
Wild type	0.99 (0.57-1.74)	0.82 (0.47-1.45)	0.92 (0.67-1.28)	0.74 (0.68-1.31)
1 or 2 variant alleles	5.62 (0.82-38.39)	13.20 (1.19-146.72)*	1.86 (0.63-5.08)	1.32 (0.68-5.98)
MTHFR C677T				
Wild type	1.78 (0.82-3.87)	1.63 (0.72-3.71)	--	--
1 or 2 variant alleles	0.83 (0.38-1.54)	0.76 (0.38-1.54)	--	--
Mothers				
*CYP2E1*5* (G1259C)				
Wild type	0.88 (0.50-1.54)	0.83 (0.48-1.44)	0.94 (0.68-1.38)	0.92 (0.66-1.28)
1 or 2 variant alleles	4.40 (0.73-26.42)	6.54 (0.59-71.45)	1.38 (0.54-3.52)	1.38 (0.54-3.53)
MTHFR C677T				
Wild type	1.00 (0.46-2.18)	0.98 (0.46-2.10)	--	--
1 or 2 variant alleles	1.12 (0.56-2.32)	0.94 (0.47-1.89)	--	--

* Chi-square (1degree of freedom) for effect modification = 4.87; p = 0.027.
Adapted from Infante-Rivard (2004).

References

Office of Environmental Health Hazard Assessment (OEHHA, 2004). Hazard Identification Document on Chloroform: Evidence on the Developmental and Reproductive Toxicity of Chloroform. http://www.oehha.ca.gov/prop65/hazard_ident/pdf_zip/ChloroformHID.pdf, California Environmental Protection Agency, OEHHA, Sacramento, CA.

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