

**Chemicals for Reproductive Toxicant (DART) Identification Committee
Review Consideration:
Particulate Matter and Trihalomethanes**

September 2008

**Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency**

Overview

At its December 2007 meeting, the Developmental and Reproductive Toxicant Identification Committee (DART IC) requested that OEHHA compile abstracts of relevant studies for particulate matter (as an air pollutant) and total trihalomethanes, using the prioritization process adopted by OEHHA in 2004. The purpose of this compilation is to provide information so that the DART IC can advise OEHHA concerning whether to develop hazard identification materials for these substances. The two substances and relevant documentation are being released to the public for a 60-day comment period in preparation for consultation with the DART IC during its November 20, 2008 meeting. At that meeting, the DART IC will recommend to OEHHA whether hazard identification materials should be prepared for these chemicals as the next step toward future consideration of their listing.

Background

At its December 2007 meeting, the DART IC reviewed information developed by OEHHA (2007) on sulfur dioxide and bromodichloromethane, along with other chemicals, to advise OEHHA on whether to develop hazard identification materials for these substances. This activity was being conducted under the prioritization process adopted by OEHHA in 2004. The Committee recommended that OEHHA develop similar materials on related substances - particulate matter (as an air pollutant) and total trihalomethanes.

The Appendix on particulate matter (as an air pollutant) and total trihalomethanes provide information on the overall evidence of reproductive or developmental toxicity for these two substances. There are numerous epidemiology studies reporting associations of exposures to these chemicals and reproductive outcomes. Such epidemiological studies and other data relevant to the reproductive or developmental toxicity of these substances are provided in papers and other materials listed in the Appendix, along with verbatim abstracts of these studies obtained from sources such as on-line journals or PubMed.

Next Steps

With the publication of this document on September 5, 2008, OEHHA opened the public comment period on the chemicals proposed for DART IC consideration. The comment period closes on November 4, 2008.

The DART IC will deliberate on these two substances proposed for its consideration at its November 20, 2008 meeting, and provide advice and consultation regarding possible development of hazard identification materials. Written public comments received by OEHHA on or before November 4, 2008, will be provided to the DART IC for review.

At the DART IC meeting, the public will be given the opportunity to comment on the chemicals being proposed for possible hazard identification materials preparation. The DART IC could vote on recommendations or provide less formal advice to OEHHA concerning which substances should be brought back for future consideration for listing. In addition, the DART IC may also suggest other substances for which hazard identification materials should be prepared.

Following the DART IC recommendations, OEHHA will select substances for the preparation of hazard identification materials. These materials will summarize the available scientific evidence on the developmental and reproductive toxicity potential of the selected chemicals, based on an exhaustive search and evaluation of the scientific literature. OEHHA will provide these materials to the DART IC and release them for public comment. The DART IC will then hold another public meeting to deliberate the listing of the chemicals.

Further details on prioritization, the development of hazard identification materials, and committee consideration of the listing of chemicals under Proposition 65 are given in the OEHHA (2004) document cited below.

References

Office of Environmental Health Hazard Assessment (OEHHA, 2004). *Process for Prioritizing Chemicals for Consideration under Proposition 65 by the "State's Qualified Experts."* California Environmental Protection Agency, OEHHA, Sacramento, CA, December. Available online at: www.oehha.ca.gov/prop65/CRNR_notices/state_listing/pdf/finalPriordoc.pdf

Office of Environmental Health Hazard Assessment (OEHHA, 2007). Chemicals Submitted to the Developmental and Reproductive Toxicant Identification Committee for Consultation: Sulfur dioxide (http://www.oehha.ca.gov/prop65/CRNR_notices/state_listing/pdf/dart090707_10.pdf); Bromodichloromethane (http://www.oehha.ca.gov/prop65/CRNR_notices/state_listing/pdf/dart090707_4.pdf)

PARTICULATE MATTER

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of the air pollutant Particulate Matter (PM). PM is of particular concern as an air pollutant, where it occurs in combination with other chemicals of concern such as sulfur dioxide and carbon monoxide.

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. The epidemiologic studies report on developmental and reproductive sequelae related to environmental exposures to particulate matter, usually in combination with other components of air pollution.

- Thirty epidemiologic studies of PM reporting increased risk of adverse developmental or reproductive outcomes were identified. Four meeting abstracts reporting increased risk of adverse developmental or reproductive outcomes were also identified. Four epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified, of which two were meeting abstracts. Twelve related articles were also identified.
- Eleven animal studies of PM reporting reproductive or developmental toxicity were identified, as well as two studies reporting no reproductive or developmental toxicity. Three related articles were also identified.

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I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

Ambient air pollution and low birth weight in Connecticut and Massachusetts.

Bell M. L., Ebisu K. and Belanger K.

Environmental Health Perspectives. 2007 115(7):1118-24.

BACKGROUND: Several studies have examined whether air pollution affects birth weight; however results vary and many studies were focused on Southern California or were conducted outside of the United States. **OBJECTIVES:** We investigated maternal exposure to particulate matter with aerodynamic diameter $< 10, < 2.5$ microm (PM(10), PM(2.5)), sulfur dioxide, nitrogen dioxide, and carbon monoxide and birth weight for 358,504 births in Massachusetts and Connecticut from 1999 to 2002. **METHODS:** Analysis included logistic models for low birth weight ($< 2,500$ g) and linear models with birth weight as a continuous variable. Exposure was assigned as the average county-level concentration over gestation and each trimester based on mother's residence. We adjusted for gestational length, prenatal care, type of delivery, child's sex, birth order, weather, year, and mother's race, education, marital status, age, and tobacco use. **RESULTS:** An interquartile increase in gestational exposure to NO(2), CO, PM(10), and PM(2.5) lowered birth weight by 8.9 g [95% confidence interval (CI), 7.0-10.8], 16.2 g (95% CI, 12.6-19.7), 8.2 g (95% CI, 5.3-11.1), and 14.7 g (95% CI, 12.3-17.1), respectively. Lower birth weight was associated with exposure in the third trimester for PM(10), the first and third trimesters for CO, the first trimester for NO(2) and SO(2), and the second and third trimesters for PM(2.5). Effect estimates for PM(2.5) were higher for infants of black mothers than those of white mothers. **CONCLUSIONS:** Results indicate that exposure to air pollution, even at low levels, may increase risk of low birth weight, particularly for some segments of the population.

Pre-pregnancy dietary vitamin A intake may alleviate the adverse birth outcomes associated with prenatal pollutant exposure: epidemiologic cohort study in Poland.

Jedrychowski W., Masters E., Choi H., Sochacka E., Flak E., Mroz E., Pac A., Jacek R., Kaim I., Skolicki Z., Spengler J. D. and Perera F.

International Journal of Occupational and Environmental Health. 2007 13(2):175-80.

A cohort study assessed the relationship between dietary intake of vitamin A in 493 healthy mothers before and around conception and adverse birth outcomes associated with environmental toxicant exposures. The cohort, non-smoking women with singleton pregnancies, aged 18-35 years, gave birth at 34-43 weeks of gestation. The women were asked about their diets over one year preceding pregnancy. Measurements of PM_{2.5} were carried out during the second trimester. Birth outcomes were adjusted for potential confounding factors, including gestational age. Standardized beta regression coefficients confirmed an inverse association between PM_{2.5} and birth weight (beta = -172.4, p = 0.02), but the effect of vitamin A on birth weight was positive (beta = 176.05, p = 0.05), when the two were adjusted for each other. The

negative effect of higher prenatal PM_{2.5} exposures (above third tertile) on birth weight was significant in women below the third tertile of vitamin A intakes (beta = -185.1, p = 0.00), but not in women with higher intakes (beta = 38.6, p = 0.61). The negative effect of higher PM_{2.5} exposure on length at birth was significant with lower vitamin A intakes (beta = -1.1, p = 0.00) but not with higher intakes (beta = -0.3, p = 0.56). Prepregnancy nutrition of mothers may modulate the harmful effects of prenatal exposures to pollutants on birth outcomes.

PM₁₀ and pregnancy outcomes: a hospital-based cohort study of pregnant women in Seoul.

Kim O. J., Ha E. H., Kim B. M., Seo J. H., Park H. S., Jung W. J., Lee B. E., Suh Y. J., Kim Y. J., Lee J. T., Kim H. and Hong Y. C.

Journal of Occupational and Environmental Medicine. 2007 49(12):1394-402.

OBJECTIVE:: The aim of this study was to evaluate the effects of PM₁₀ on birth outcomes using a prospective cohort of pregnant women. **METHODS::** The multicenter prospective study was conducted in Korea from 2001 to 2004. To estimate the effects of PM₁₀ exposure on birth outcomes, the logistic and linear regression model and the generalized additive model for nonlinear relationships were used. **RESULTS::** Stillbirths were affected by PM₁₀ level during the third trimesters (OR = 1.10, 95% CI = 1.02-1.14), and birth defects were influenced by the PM₁₀ exposure during the second trimesters (OR = 1.16, 95% CI = 1.00-1.34). Intrauterine growth retardation was affected by the first trimester's PM₁₀ exposure. On the other hand, premature birth was affected by the PM₁₀ exposure during the third trimester, and low-birth-weight births were affected by the PM₁₀ level during entire trimesters of pregnancy. **CONCLUSIONS::** PM₁₀ exposure during pregnancy may result in adverse birth outcomes with different critical periods.

Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles.

Ritz B., Wilhelm M., Hoggatt K. J. and Ghosh J. K.

American Journal of Epidemiology. 2007 1;166(9):1045-52.

The authors conducted a case-control survey nested within a birth cohort and collected detailed risk factor information to assess the extent to which residual confounding and exposure misclassification may impact air pollution effect estimates. Using a survey of 2,543 of 6,374 women sampled from a cohort of 58,316 eligible births in 2003 in Los Angeles County, California, the authors estimated with logistic regression and two-phase models the effects of pregnancy period-specific air pollution exposure on the odds of preterm birth. For the first trimester, the odds of preterm birth consistently increased with increasing carbon monoxide exposures and also at high levels of exposure to particulate matter less than or equal to 2.5 microm in diameter (>21.4 microg/m³), regardless of type of data (cohort/sample) or covariate adjustment (carbon monoxide exposures of >1.25 ppm increased the odds by 21-25%). Women exposed to carbon monoxide above 0.91 ppm during the last 6 weeks of pregnancy experienced increased odds of preterm birth. Crude and birth certificate covariate-adjusted results for carbon monoxide differed from each other. However, further adjustment for risk factors assessed in the survey did not change effect estimates for short-term pollutant averages appreciably, except for

time-activity patterns, which strengthened the observed associations. These results confirm the importance of reducing exposure misclassification when evaluating the effect of traffic-related pollutants that vary spatially.

Traffic-related atmospheric pollutants levels during pregnancy and offspring's term birth weight: a study relying on a land-use regression exposure model.

Slama R., Morgenstern V., Cyrus J., Zutavern A., Herbarth O., Wichmann H. E., Heinrich J. and Lisa S. G.

Environmental Health Perspectives. 2007 115(9):1283-92.

BACKGROUND: Some studies have suggested that particulate matter (PM) levels during pregnancy may be associated with birth weight. Road traffic is a major source of fine PM (PM with aerodynamic diameter < 2.5 microm; PM(2.5)). **OBJECTIVE:** We determined to characterize the influence of maternal exposure to atmospheric pollutants due to road traffic and urban activities on offspring term birth weight. **METHODS:** Women from a birth cohort [the LISA (Influences of Lifestyle Related Factors on the Human Immune System and Development of Allergies in Children) cohort] who delivered a non-premature baby with a birth weight > 2,500 g in Munich metropolitan area were included. We assessed PM(2.5), PM(2.5) absorbance (which depends on the blackness of PM(2.5), a marker of traffic-related air pollution), and nitrogen dioxide levels using a land-use regression model, taking into account the type and length of roads, population density, land coverage around the home address, and temporal variations in pollution during pregnancy. Using Poisson regression, we estimated prevalence ratios (PR) of birth weight < 3,000 g, adjusted for gestational duration, sex, maternal smoking, height, weight, and education. **RESULTS:** Exposure was defined for 1,016 births. Taking the lowest quartile of exposure during pregnancy as a reference, the PR of birth weight < 3,000 g associated with the highest quartile was 1.7 for PM(2.5) [95% confidence interval (CI), 1.2-2.7], 1.8 for PM(2.5) absorbance (95% CI, 1.1-2.7), and 1.2 for NO₂ (95% CI, 0.7-1.7). The PR associated with an increase of 1 microg/m³ in PM(2.5) levels was 1.13 (95% CI, 1.00-1.29). **CONCLUSION:** Increases in PM(2.5) levels and PM(2.5) absorbance were associated with decreases in term birth weight. Traffic-related air pollutants may have adverse effects on birth weight.

Cytochrome P450IA1 polymorphisms along with PM(10) exposure contribute to the risk of birth weight reduction.

Suh Y. J., Kim B. M., Park B. H., Park H., Kim Y. J., Kim H., Hong Y. C. and Ha E. H.
Reproductive Toxicology. 2007 24(3-4):281-8.

We explored the effects of particulate matter <10 microm (PM(10)) exposure along with CYP1A1 polymorphisms of MspI (T6235C) and NcoI (Ile462Val) on reduced birth weight (BW). A prospective cohort study was done with women who delivered from 2001 to 2004 at Ewha Womans University Hospital, Seoul, Korea. We compared the estimated least squares means of BW in the generalized linear model, after adjusting for controlling factors. High PM(10) exposure at the 90th percentile level and above during the 1st trimester conferred a significant risk for reduced BW, compared with low PM(10) exposure below the 90th percentile

level. The effect of high PM(10) exposure during the 1st trimester of pregnancy compared with low PM(10) exposure was greater for women with MspI TC/CC and NcoI IleVal/ValVal genotypes than for those with MspI TT and NcoI IleIle genotypes. In conclusion, high PM(10) exposure during the 1st trimester increased the risk for reduced BW in concert with MspI TC/CC and NcoI IleVal/ValVal genotypes in Korean women.

The association between low level exposures to ambient air pollution and term low birth weight: a retrospective cohort study.

Dugandzic R., Dodds L., Stieb D. and Smith-Doiron M.
Environmental Health. 2006 5:3.

BACKGROUND: Studies in areas with relatively high levels of air pollution have found some positive associations between exposures to ambient levels of air pollution and several birth outcomes including low birth weight (LBW). The purpose of this study was to examine the association between LBW among term infants and ambient air pollution, by trimester of exposure, in a region of lower level exposures. **METHODS:** The relationship between LBW and ambient levels of particulate matter up to 10 um in diameter (PM10), sulfur dioxide (SO₂) and ground-level ozone (O₃) was evaluated using the Nova Scotia Atlee Perinatal Database and ambient air monitoring data from the Environment Canada National Air Pollution Surveillance Network and the Nova Scotia Department of Environment. The cohort consisted of live singleton births (> or =37 weeks of gestation) between January 1, 1988 and December 31, 2000. Maternal exposures to air pollution were assigned to women living within 25 km of a monitoring station at the time of birth. Air pollution was evaluated as a continuous and categorical variable (using quartile exposures) for each trimester and relative risks were estimated from logistic regression, adjusted for confounding variables. **RESULTS:** There were 74,284 women with a term, singleton birth during the study period and with exposure data. In the analyses unadjusted for year of birth, first trimester exposures in the highest quartile for SO₂ and PM10 suggested an increased risk of delivering a LBW infant (relative risk = 1.36, 95% confidence interval = 1.04 to 1.78 for SO₂ exposure and relative risk = 1.33, 95% confidence interval = 1.02 to 1.74 for PM10). After adjustment for birth year, the relative risks were attenuated somewhat and not statistically significant. A dose-response relationship for SO₂ was noted with increasing levels of exposure. No statistically significant effects were noted for ozone. **CONCLUSION:** Our results suggest that exposure during the first trimester to relatively low levels of some air pollutants may be associated with a reduction in birth weight in term-born infants. These findings have implications for the development of effective risk management strategies to minimize the public health impacts for pregnant women.

Maternal exposure to low levels of ambient air pollution and preterm birth in Brisbane, Australia.

Hansen C., Neller A., Williams G. and Simpson R.
BJOG. 2006 113(8):935-41.

BCKGROUND: There is evidence that maternal exposure to ambient air pollution during pregnancy is associated with adverse birth outcomes. **OBJECTIVE:** To assess preterm birth (PTB) in relation to maternal exposure to ambient air pollution. **DESIGN:** Retrospective cohort. **SETTING:** Brisbane, Australia. **POPULATION:** A total of 28,200 singleton live births for the period of 1 July 2000 to 30 June 2003. **METHODS:** Average maternal exposure estimates for ambient particulate matter (PM(10) and bsp), ozone (O(3)) and nitrogen dioxide were calculated over the first 3 months after last menstrual period (LMP) and the last 3 months prior to birth (individually and combined as trimesters). **MAIN OUTCOME MEASURES:** PTB was defined as gestation <37 weeks and odds ratios (OR) were calculated for PTB per interquartile range increase in the maternal exposure estimate for each pollutant. Various covariates were controlled for, including season of birth. **RESULTS:** Exposure to PM(10) and O(3) during trimester one was associated with an increased risk of PTB (OR = 1.15, 95% CI 1.06-1.25 and OR = 1.26, 95% CI 1.10-1.45, respectively). The PM(10) exposure effect associated with trimester one was strongly related to exposure during the first month post-LMP (PM(10), month one; OR = 1.19, 95% CI 1.13-1.26). **CONCLUSION:** These results suggest that maternal exposure to low levels of ambient air pollution is associated with PTB.

Relationships between air pollution and preterm birth in California.

Huynh M., Woodruff T. J., Parker J. D. and Schoendorf K. C.
Paediatric and Perinatal Epidemiology. 2006 20(6):454-61.

Air pollution from vehicular emissions and other combustion sources is related to cardiovascular and respiratory outcomes. However, few studies have investigated the relationship between air pollution and preterm birth, a primary cause of infant mortality and morbidity. This analysis examined the effect of fine particulate matter (PM(2.5)) and carbon monoxide (CO) on preterm birth in a matched case-control study. PM(2.5) and CO monitoring data from the California Air Resources Board were linked to California birth certificate data for singletons born in 1999-2000. Each birth was mapped to the closest PM monitor within 5 miles of the home address. County-level CO measures were utilised to increase sample size and maintain a representative population. After exclusion of implausible birthweight-gestation combinations, preterm birth was defined as birth occurring between 24 and 36 weeks' gestation. Each of the 10 673 preterm cases was matched to three controls of term (39-44 weeks) gestation with a similar date of last menstrual period. Based on the case's gestational age, CO and PM(2.5) exposures were calculated for total pregnancy, first month of pregnancy, and last 2 weeks of pregnancy. Exposures were divided into quartiles; the lowest quartile was the reference. Because of the matched design, conditional logistic regression was used to adjust for maternal race/ethnicity, age, parity, marital status and education. High total pregnancy PM(2.5) exposure was associated with a small effect on preterm birth, after adjustment for maternal factors (adjusted odds ratio [AOR] = 1.15, [95% CI 1.07, 1.24]). The odds ratio did not change after adjustment for CO.

Results were similar for PM(2.5) exposure during the first month of pregnancy (AOR = 1.21, 95% CI [1.12, 1.30]) and the last 2 weeks of pregnancy (AOR = 1.17, 95% CI [1.09, 1.27]). Conversely, CO exposure at any time during pregnancy was not associated with preterm birth (AORs from 0.95 to 1.00). Maternal exposure to PM(2.5), but not CO, is associated with preterm birth. This analysis did not show differences by timing of exposure, although more detailed examination may be needed.

Exposures to air pollutants during pregnancy and preterm delivery.

Leem J. H., Kaplan B. M., Shim Y. K., Pohl H. R., Gotway C. A., Bullard S. M., Rogers J. F., Smith M. M. and Tylenda C. A.
Environmental Health Perspectives. 2006 114(6):905-10.

The association between preterm delivery (PTD) and exposure to air pollutants has recently become a major concern. We investigated this relationship in Incheon, Republic of Korea, using spatial and temporal modeling to better infer individual exposures. The birth cohort consisted of 52,113 singleton births in 2001-2002, and data included residential address, gestational age, sex, birth date and order, and parental age and education. We used a geographic information system and kriging methods to construct spatial and temporal exposure models. Associations between exposure and PTD were evaluated using univariate and multivariate log-binomial regressions. Given the gestational age, birth date, and the mother's residential address, we estimated each mother's potential exposure to air pollutants during critical periods of the pregnancy. The adjusted risk ratios for PTD in the highest quartiles of the first trimester exposure were 1.26 [95% confidence interval (CI), 1.11-1.44] for carbon monoxide, 1.27 (95% CI, 1.04-1.56) for particulate matter with aerodynamic diameter ≤ 10 microm, 1.24 (95% CI, 1.09-1.41) for nitrogen dioxide, and 1.21 (95% CI, 1.04-1.42) for sulfur dioxide. The relationships between PTD and exposures to CO, NO₂, and SO₂ were dose dependent ($p < 0.001$, $p < 0.02$, $p < 0.02$, respectively). In addition, the results of our study indicated a significant association between air pollution and PTD during the third trimester of pregnancy. In conclusion, our study showed that relatively low concentrations of air pollution under current air quality standards during pregnancy may contribute to an increased risk of PTD. A biologic mechanism through increased prostaglandin levels that are triggered by inflammatory mediators during exposure periods is discussed.

Air pollution and very low birth weight infants: a target population?

Rogers J. F. and Dunlop A. L.
Pediatrics. 2006 118(1):156-64.

OBJECTIVE: The goal was to examine systematically the association between maternal exposure to particulate matter of <10 microm and very low birth weight (<1500 g) delivery for evidence of an effect on duration of gestation and/or intrauterine growth restriction. **METHODS:** This case-control study took place between April 1, 1986, and March 30, 1988, in Georgia Health Care District 9 and included 128 mothers of very low birth weight infants, all of whom were preterm and were classified as either small for gestational age or appropriate for gestational age, and 197 mothers of term, appropriate-for-gestational-age infants weighing ≥ 2500 g.

Maternal exposure to particulate matter of <10 microm was estimated with 2 exposure measures, namely, a county-level measure based on residence in a county with an industrial point source and an environmental transport model based on the geographic location of the birth home.

RESULTS: Considering preterm/appropriate-for-gestational-age infants as cases and term/appropriate-for-gestational-age infants as controls, adjusted odds ratios for maternal exposure to particulate matter of <10 microm were statistically significant (adjusted odds ratio for county-level model: 4.31; adjusted odds ratio for environmental transport model: 3.68). Although elevated, no statistically significant association was found between maternal exposure and preterm/appropriate-for-gestational-age delivery when compared to preterm/small-for-gestational-age delivery. **CONCLUSIONS:** There are increased odds of maternal exposure to ambient particulate matter of <10 microm for very low birth weight preterm/appropriate-for-gestational-age delivery, compared with term/appropriate-for-gestational-age delivery, which suggests that the observed association between maternal exposure to air pollution and low infant birth weight (particularly <1500 g) is at least partially attributable to an effect on duration of gestation.

Relation between ambient air quality and selected birth defects, seven county study, Texas, 1997-2000.

Gilboa S. M., Mendola P., Olshan A. F., Langlois P. H., Savitz D. A., Loomis D., Herring A. H. and Fixler D. E.
American Journal of Epidemiology. 2005 162(3):238-52.

A population-based case-control study investigated the association between maternal exposure to air pollutants, carbon monoxide, nitrogen dioxide, ozone, sulfur dioxide, and particulate matter <10 microm in aerodynamic diameter during weeks 3-8 of pregnancy and the risk of selected cardiac birth defects and oral clefts in livebirths and fetal deaths between 1997 and 2000 in seven Texas counties. Controls were frequency matched to cases on year of birth, vital status, and maternal county of residence at delivery. Stationary monitoring data were used to estimate air pollution exposure. Logistic regression models adjusted for covariates available in the vital record. When the highest quartile of exposure was compared with the lowest, the authors observed positive associations between carbon monoxide and tetralogy of Fallot (odds ratio = 2.04, 95% confidence interval: 1.26, 3.29), particulate matter <10 microm in aerodynamic diameter and isolated atrial septal defects (odds ratio = 2.27, 95% confidence interval: 1.43, 3.60), and sulfur dioxide and isolated ventricular septal defects (odds ratio = 2.16, 95% confidence interval: 1.51, 3.09). There were inverse associations between carbon monoxide and isolated atrial septal defects and between ozone and isolated ventricular septal defects. Evidence that air pollution exposure influences the risk of oral clefts was limited. Suggestive results support a previously reported finding of an association between ozone exposure and pulmonary artery and valve defects.

Impact of ambient air pollution on birth weight in Sydney, Australia.

Mannes T., Jalaludin B., Morgan G., Lincoln D., Sheppard V. and Corbett S.
Occupational and Environmental Medicine. 2005 62(8):524-30.

BACKGROUND: Studies in Asia, Europe, and the Americas have provided evidence that ambient air pollution may have an adverse effect on birth weight, although results are not consistent. **METHODS:** Average exposure during pregnancy to five common air pollutants was estimated for births in metropolitan Sydney between 1998 and 2000. The effects of pollutant exposure in the first, second, and third trimesters of pregnancy on risk of "small for gestational age" (SGA), and of pollutant exposure during pregnancy on birth weight were examined. **RESULTS:** There were 138,056 singleton births in Sydney between 1998 and 2000; 9.7% of babies (13,402) were classified as SGA. Air pollution levels in Sydney were found to be quite low. In linear regression models carbon monoxide and nitrogen dioxide concentrations in the second and third trimesters had a statistically significant adverse effect on birth weight. For a 1 part per million increase in mean carbon monoxide levels a reduction of 7 (95% CI -5 to 19) to 29 (95% CI 7 to 51) grams in birth weight was estimated. For a 1 part per billion increase in mean nitrogen dioxide levels a reduction of 1 (95% CI 0 to 2) to 34 (95% CI 24 to 43) grams in birth weight was estimated. Particulate matter (diameter less than ten microns) in the second trimester had a small statistically significant adverse effect on birth weight. For a 1 microgram per cubic metre increase in mean particulate matter levels a reduction of 4 grams (95% CI 3 to 6) in birth weight was estimated. **CONCLUSION:** These findings of an association between carbon monoxide, nitrogen dioxide, and particulate matter, and reduction in birth weight should be corroborated by further study.

Air pollution and birth weight among term infants in California.

Parker J. D., Woodruff T. J., Basu R. and Schoendorf K. C.
Pediatrics. 2005 115(1):121-8.

OBJECTIVE: To examine associations between birth weight and air pollution among full-term infants in California. **METHODS:** We matched exposure data collected from air pollution monitors for small particles (PM(2.5)) and carbon monoxide (CO) to California birth records for singleton births delivered at 40 weeks' gestation in 2000 using the locations of the monitors and mother's residence. Pollution measurements collected within 5 miles of the mother's residence, averaged for the time period corresponding to the duration of pregnancy and each trimester, were used as exposure variables. Logistic and linear regression models were used to estimate the associations between the pollution measures and 2 pregnancy outcomes: small for gestational age (SGA) and birth weight. Variations of the models were used to examine the robustness of the findings. **RESULTS:** The adjusted odds ratio for SGA for exposure in the highest compared with lowest quartile of PM(2.5) was 1.26 (95% confidence interval [CI]: 1.03-1.50). We found no association between CO and birth weight or SGA after controlling for maternal factors and PM(2.5) (mean birth weight difference: 2.6 g; 95% CI: -20.6 to 25.8). The difference in mean birth weight for infants with a 9-month exposure in the highest quartile of PM(2.5) compared with that of infants who were exposed in the lowest quartile was -36.1 g (95% CI: -16.5 g to -55.8 g); this difference was similar after controlling for CO. We did not find PM(2.5) exposure

during a particular trimester most important for assessing birth weight; trimester-level associations were similar to those found using the 9-month exposure variable. **CONCLUSIONS:** We found an increased odds of SGA and a small difference in mean birth weight between infants with the highest and lowest exposures to PM(2.5) but not CO. These findings have important implications for infant health because of the ubiquitous exposure to fine particulate air pollution across the United States.

A time-series analysis of air pollution and preterm birth in Pennsylvania, 1997-2001.

Sagiv S. K., Mendola P., Loomis D., Herring A. H., Neas L. M., Savitz D. A. and Poole C. Environmental Health Perspectives. 2005 113(5):602-6.

Preterm delivery can lead to serious infant health outcomes, including death and lifelong disability. Small increases in preterm delivery risk in relation to spatial gradients of air pollution have been reported, but previous studies may have controlled inadequately for individual factors. Using a time-series analysis, which eliminates potential confounding by individual risk factors that do not change over short periods of time, we investigated the effect of ambient outdoor particulate matter with diameter ≤ 10 microm (PM10) and sulfur dioxide on risk for preterm delivery. Daily counts of preterm births were obtained from birth records in four Pennsylvania counties from 1997 through 2001. We observed increased risk for preterm delivery with exposure to average PM10 and SO2 in the 6 weeks before birth [respectively, relative risk (RR) = 1.07; 95% confidence interval (CI), 0.98-1.18 per 50 microg/m³ increase; RR = 1.15; 95% CI, 1.00-1.32 per 15 ppb increase], adjusting for long-term preterm delivery trends, co-pollutants, and offsetting by the number of gestations at risk. We also examined lags up to 7 days before the birth and found an acute effect of exposure to PM10 2 days and 5 days before birth (respectively, RR = 1.10; 95% CI, 1.00-1.21; RR = 1.07; 95% CI, 0.98-1.18) and SO2 3 days before birth (RR = 1.07; 95% CI, 0.99-1.15), adjusting for covariates, including temperature, dew point temperature, and day of the week. The results from this time-series analysis, which provides evidence of an increase in preterm birth risk with exposure to PM10 and SO2, are consistent with prior investigations of spatial contrasts.

Local variations in CO and particulate air pollution and adverse birth outcomes in Los Angeles County, California, USA.

Wilhelm M. and Ritz B.

Environmental Health Perspectives. 2005 113(9):1212-21.

We extended our previous analyses of term low birth weight (LBW) and preterm birth to 1994-2000, a period of declining air pollution levels in the South Coast Air Basin. We speculated that the effects we observed previously for carbon monoxide, particulate matter < 10 microm in aerodynamic diameter (PM10), and traffic density were attributable to toxins sorbed to primary exhaust particles. Focusing on CO, PM10, and particulate matter < 2.5 microm in aerodynamic diameter (PM2.5), we examined whether varying residential distances from monitoring stations affected risk estimates, because effect attenuation may result from local pollutant heterogeneity inadequately captured by ambient stations. We geocoded home locations, calculated the distance to the nearest air monitors, estimated exposure levels by pregnancy period, and performed

logistic regression analyses for subjects living within 1-4 mi of a station. For women residing within a 1-mi distance, we observed a 27% increase in risk for high (> or = 75th percentile) first-trimester CO exposures and preterm birth and a 36% increase for high third-trimester pregnancy CO exposures and term LBW. For particles, we observed similar size effects during early and late pregnancy for both term LBW and preterm birth. In contrast, smaller or no effects were observed beyond a 1-mi distance of a residence from a station. Associations between CO and PM10 averaged over the whole pregnancy and term LBW were generally smaller than effects for early and late pregnancy. These new results for 1994-2000 generally confirm our previous observations for the period 1989-1993, again linking CO and particle exposures to term LBW and preterm birth. In addition, they confirm our suspicions about having to address local heterogeneity for these pollutants in Los Angeles.

Association between ambient air pollution and birth weight in Sao Paulo, Brazil.

Gouveia N., Bremner S. A. and Novaes H. M.

Journal of Epidemiology and Community Health. 2004 58(1):11-7.

OBJECTIVES: Previous studies have implicated air pollution in increased mortality and morbidity, especially in the elderly population and children. More recently, associations with mortality in infants and with some reproductive outcomes have also been reported. The aim of this study is to explore the association between exposure to outdoor air pollution during pregnancy and birth weight. **DESIGN:** Cross sectional study using data on all singleton full term live births during a one year period. For each individual birth, information on gestational age, type of delivery, birth weight, sex, maternal education, maternal age, place of residence, and parity was available. Daily mean levels of PM(10), sulphur dioxide, nitrogen dioxide, carbon monoxide, and ozone were also gathered. The association between birth weight and air pollution was assessed in regression models with exposure averaged over each trimester of pregnancy. **SETTING:** Sao Paulo city, Brazil. **RESULTS:** Birth weight was shown to be associated with length of gestation, maternal age and instruction, infant gender, number of antenatal care visits, parity, and type of delivery. On adjusting for these variables negative effects of exposure to PM(10) and carbon monoxide during the first trimester were observed. This effect seemed to be more robust for carbon monoxide. For a 1 ppm increase in mean exposure to carbon monoxide during the first trimester a reduction of 23 g in birth weight was estimated. **CONCLUSIONS:** The results are consistent in revealing that exposure to air pollution during pregnancy may interfere with weight gain in the fetus. Given the poorer outlook for low birthweight babies on a number of health outcomes, this finding is important from the public health perspective.

Estimated risk for altered fetal growth resulting from exposure to fine particles during pregnancy: an epidemiologic prospective cohort study in Poland.

Jedrychowski W., Bendkowska I., Flak E., Penar A., Jacek R., Kaim I., Spengler J. D., Camann D. and Perera F. P.

Environmental Health Perspectives. 2004 112(14):1398-402.

The purpose of this study was to estimate exposure of pregnant women in Poland to fine particulate matter [less than or equal to 2.5 microm in diameter (PM 2.5)] and to assess its effect

on the birth outcomes. The cohort consisted of 362 pregnant women who gave birth between 34 and 43 weeks of gestation. The enrollment included only nonsmoking women with singleton pregnancies, 18-35 years of age, who were free from chronic diseases such as diabetes and hypertension. PM 2.5 was measured by personal air monitoring over 48 hr during the second trimester of pregnancy. All assessed birth effects were adjusted in multiple linear regression models for potential confounding factors such as the size of mother (maternal height, prepregnancy weight), parity, sex of child, gestational age, season of birth, and self-reported environmental tobacco smoke (ETS). The regression model explained 35% of the variability in birth weight (beta = -200.8, p = 0.03), and both regression coefficients for PM 2.5 and birth length (beta = -1.44, p = 0.01) and head circumference (HC; beta = -0.73, p = 0.02) were significant as well. In all regression models, the effect of ETS was insignificant. Predicted reduction in birth weight at an increase of exposure from 10 to 50 microg/m³ was 140.3 g. The corresponding predicted reduction of birth length would be 1.0 cm, and of HC, 0.5 cm. The study provides new and convincing epidemiologic evidence that high personal exposure to fine particles is associated with adverse effects on the developing fetus. These results indicate the need to reduce ambient fine particulate concentrations. However, further research should establish possible biologic mechanisms explaining the observed relationship.

Investigating adverse birth outcomes and air pollution in Michigan.

Sadeghnejad A., Hultin M. L., Batterman S., Depa M., Wirth J. and Wahl R. L. *Epidemiology* 2004;15(4).

Introduction: A growing body of literature indicates that exposure to ambient levels of air pollutants adversely affects fetal development. Exposure to the criteria air pollutants carbon monoxide (CO), nitrogen dioxide (NO₂), ozone (O₃), particulate matter less than 2.5 m (PM_{2.5}) and sulfur dioxide (SO₂) have been associated with low birth weight, premature birth and small for gestational aged neonates. To assess the potential effects of these pollutants, a semi-individual study was designed to merge databases containing levels of criteria air pollutants measured at four air monitors in two areas of Michigan with a database containing individual level birth certificate information. Methods: Birth certificate data from 1990-2001 was obtained on births among residents of 36 zip codes in Allen Park (AP), Lansing (LA) and in East Seven Mile (E7) and Linwood (LI) in Detroit. Zip codes within a 4- kilometer radius of an air monitoring station providing data on levels of CO, NO₂, O₃, PM_{2.5}, SO₂, and meteorological assessments were included. Among infants of 16-45 year old mothers, singletons weighing 750-4000g with a gestational age of 20-40 weeks were included. The primary adverse birth outcome is low birth weight (LBW, < 1500g). LBW risk factors in the study included maternal race, age, prenatal care level (Kessner Index), site of residence, history of certain diseases, and smoking, as well as gender of the newborn and year and month of the delivery. Results: Analyses included 207,481 newborns (80% of the initial sample). The ratio of African-American/Caucasian and mean birth weight was 6/94 and 3245g in AP, 76/24 and 3048g in LI, 65/35 and 3094g in E7, and 20/80 and 3212g in LA, respectively. Mean gestational age was approximately 37 weeks at all sites. Risk factors (OR and 95% CI > 1.0) for LBW were low gestational age (< 38 weeks), maternal diseases (except for diabetes), maternal smoking, African-American ethnicity of the mother, female newborn, age of the mother (< 20 years and > 30 years) and the site of residence

(others versus AP). Discussion: In accordance with previous studies, the analysis indicated that low birth weight is partially explained by maternal health conditions, habits, and ethnicity as well as newborn's gestational age and gender. Several regression models for each pollutant as the exposure variable and controlling for LBW risk factors are being examined. In the final model, we will include all pollutants that show a significant effect in single pollutant models.

Air pollution and risk for preterm birth in Pennsylvania, 1997-2001: A time series approach.

Sagiv S., Mendola P., Loomis D., Herring A., Neas L., Savitz D. and Poole C.
American Journal of Epidemiology. 2004 159(11 Suppl).

Preterm delivery can lead to serious infant health outcomes including death and life-long disability. Small increases in preterm delivery risk in relation to air pollution have been reported, but prior investigations may have inadequately controlled for individual factors, such as smoking. We conducted a time-series analysis, which eliminates confounding by individual factors, to investigate the effect of ambient outdoor particulate matter with diameter of ≤ 10 micrometers (PM10) and sulfur dioxide (SO₂) on risk for preterm delivery. Daily counts of preterm births were obtained from birth records in four Pennsylvania counties between 1997 and 2001. PM10 and SO₂ levels were averaged over a six-week window directly preceding birth. Full, adjusted models controlled for long-term seasonal trends, co-pollutants (nitrogen dioxide, carbon monoxide, SO₂, PM10), and were offset by the number of live births in the population. County-level information was incorporated using a mixed model with a random intercept. Of 187,997 singleton births, 21,450 (11.4%) were born preterm. In full, adjusted models, we observed approximately 1 excess preterm birth for every 100 births exposed to a 50 $\mu\text{g}/\text{m}^3$ increase in PM10 (Risk Ratio = 1.09; 95% CI: 1.01, 1.19) and 2 excess preterm births for every 100 births exposed to a 15 parts per billion (ppb) SO₂ increase (RR = 1.20%, 95% CI: 1.04, 1.39). While the absolute increases in risk observed here are small, they may have important public health impact due to the high prevalence of preterm birth.

Exposure to air pollution during different gestational phases contributes to risks of low birth weight.

Lee B. E., Ha E. H., Park H. S., Kim Y. J., Hong Y. C., Kim H. and Lee J. T.
Human Reproduction. 2003 18(3):638-43.

BACKGROUND: Although there have been growing concerns about the adverse effects of air pollution on birth outcomes, little is known about which specific exposure times of specific pollutants contribute to low birth weight (LBW). **METHODS:** We evaluated the relationships between LBW and air pollution exposure levels in Seoul, Korea. Using the air pollution data, we estimated the exposure during each trimester and also during each month of pregnancy on the basis of the gestational age and birth date of each newborn. Generalized additive logistic regression analyses were conducted considering infant sex, birth order, maternal age, parental education level, time trend, and gestational age. **RESULTS:** The monthly analyses suggested that the risks for LBW tended to increase with carbon monoxide (CO) exposure between months 2-5 of pregnancy, with exposure to particles <10 micro m (PM(10)) in months 2 and 4, and for

sulphur dioxide (SO₂) and nitrogen dioxide (NO₂) exposure between months 3-5.
CONCLUSIONS: This study suggests that exposure to CO, PM₁₀, SO₂ and NO₂ during early to mid pregnancy contribute to risks for LBW.

Effects of air pollution on birth weight among children born between 1995 and 1997 in Kaohsiung, Taiwan.

Yang C. Y., Tseng Y. T. and Chang C. C.

Journal of Toxicology and Environmental Health. 2003 66(9):807-16.

Recent studies have suggested that exposure to air pollution might be associated with low birth weight. The effects of sulfur dioxide (SO₂) and particulate matter less than 10 microm (PM₁₀) were examined on birth weight in each trimester of pregnancy. The study group included all full-term singleton live births during 1995-1997 to women living within about 2 km of an air pollution monitoring site in Kaohsiung. Measurements of SO₂ and PM₁₀ collected at six air quality monitoring stations were used to estimate the influence of exposures on different pregnancy trimesters. This was done by averaging daily ambient air pollution concentrations during the corresponding days based on the birth date and gestational age of each child. Multiple linear regression analysis was used to estimate the effects of air pollution on birth weight adjusting for possible confounders including maternal age, season, marital status, maternal education, and infant gender. The estimated reduction in birth weight was 0.52 g for 1 microg/m³ increase in either SO₂ or PM₁₀ in the first trimester of pregnancy. Data provide further support for the hypothesis that air pollution can affect the outcome of pregnancy.

Air pollution and birth weight in northern Nevada, 1991-1999.

Chen L., Yang W., Jennison B. L., Goodrich A. and Omaye S. T.

Inhalation Toxicology. 2002 14(2):141-57.

This study examined the association between particulate matter < or =10 microm in aerodynamic size (PM₁₀), carbon monoxide (CO), and ozone (O₃), and birth weight in Washoe County, NV, from 1991 through 1999. In total, 39,338 single births were included in this study. The mean birth weight was 3383 +/- 460 g and prevalence of low birth weight (LBW) was 2.46% for single births with a gestational age of 37-44 wk. After controlling for cofactors including infant sex, maternal residential city, education, medical risk factors, active tobacco use, drug use, alcohol use, prenatal care, mother's age, race and ethnicity of mothers, and weight gain of mothers, we found PM₁₀ exposure in the third trimester of pregnancy to be a significant predictor of birth weight of newborns. A 10-microg/m³ increase in the 24-h PM₁₀ level in the third trimester of pregnancy can be associated with a birth-weight reduction of 11 g (95% CI: 2.3-19.8 g) using multiple linear regression; however, PM₁₀ was not found to be related with the risk of LBW from logistic regression. CO and O₃ were not found to be associated with birth weight or risk of LBW of newborns by the same modeling procedure. The results suggest PM₁₀ could be a risk factor associated with birth-weight reduction of newborns in urban northern Nevada; however, the current level of PM₁₀ is not a risk factor to increase the chance of LBW newborns.

Ambient air pollution and risk of birth defects in Southern California.

Ritz B., Yu F., Fruin S., Chapa G., Shaw G. M. and Harris J. A.
American Journal of Epidemiology. 2002 155(1):17-25.

The authors evaluated the effect of air pollution on the occurrence of birth defects ascertained by the California Birth Defects Monitoring Program in neonates and fetuses delivered in southern California in 1987-1993. By using measurements from ambient monitoring stations of carbon monoxide (CO), nitrogen dioxide, ozone, and particulate matter <10 microm in aerodynamic diameter, they calculated average monthly exposure estimates for each pregnancy. Conventional, polytomous, and hierarchical logistic regression was used to estimate odds ratios for subgroups of cardiac and orofacial defects. Odds ratios for cardiac ventricular septal defects increased in a dose-response fashion with increasing second-month CO exposure (odds ratio (OR)(2nd quartile) CO = 1.62, 95% confidence interval (CI): 1.05, 2.48; OR(3rd quartile) CO = 2.09, 95% CI: 1.19, 3.67; OR(4th quartile) CO = 2.95, 95% CI: 1.44, 6.05). Similarly, risks for aortic artery and valve defects, pulmonary artery and valve anomalies, and conotruncal defects increased with second-month ozone exposure. The study was inconclusive for other air pollutants. The authors' results are supported by the specificity of the timing of the effect and some evidence from animal data; however, this is the first known study to link ambient air pollution during a vulnerable window of development to human malformations. Confirmation by further studies is needed.

Relation between ambient air pollution and low birth weight in the Northeastern United States.

Maisonet M., Bush T. J., Correa A. and Jaakkola J. J.
Environmental Health Perspectives. 2001 109 (Suppl 3):351-6.

We evaluated the relation between term low birth weight (LBW) and ambient air levels of carbon monoxide (CO), particulate matter up to 10 microm in diameter (PM(10)), and sulfur dioxide (SO(2)). The study population consisted of singleton, term live births (37-44 weeks of gestation) born between 1 January 1994 and 31 December 1996 in six northeastern cities of the United States: Boston, Massachusetts; Hartford, Connecticut; Philadelphia, Pennsylvania; Pittsburgh, Pennsylvania; Springfield, Massachusetts; and Washington, DC. Birth data were obtained from National Center for Health Statistics Natality Data Sets. Infants with a birth weight < 2,500 g were classified as LBW. Air monitoring data obtained from the U.S. Environmental Protection Agency were used to estimate average trimester exposures to ambient CO, PM(10), and SO(2). Our results suggest that exposures to ambient CO and SO(2) increase the risk for term LBW. This risk increased by a unit increase in CO third trimester average concentration [adjusted odds ratio (AOR) 1.31; 95% confidence interval (CI) 1.06,1.62]. Infants with SO(2) second trimester exposures falling within the 25 and < 50th (AOR 1.21; CI 1.07,1.37), the 50 to < 75th (AOR 1.20; CI 1.08,1.35), and the 75 to < 95th (AOR 1.21; CI 1.03,1.43) percentiles were also at increased risk for term LBW when compared to those in the reference category (< 25th percentile). There was no indication of a positive association between prenatal exposures to PM(10) and term LBW. Increased ambient levels of air pollution may be associated with an increased risk for LBW.

Health risk assessment of urban suspended particulate matter with special reference to polycyclic aromatic hydrocarbons: a review.

Ravindra, Mittal A. K. and Van Grieken R.

Reviews on Environmental Health. 2001 16(3):169-89.

Airborne suspended particulate matter is an important marker of air quality. The term 'particulates' includes organic and inorganic matter, nitrogen compounds, sulphur compounds, polycyclic aromatic hydrocarbons (PAHs), several heavy metals, and radionuclides. The health risks from the 'classic' pollutants sulfur dioxide, nitrogen dioxide, ozone, carbon monoxide, and particulates have been comprehensively reviewed. Alarming levels of non-classic pollutants like the PAHs have been reported globally. PAHs have been found in placental tissues of women and in umbilical cord blood samples from newborn babies. The damaged DNA in cord blood is a indication of the fate of these pollutants in the environment. Hence, a need exists for a comprehensive investigation of the human health-related aspects of exposure to particulates and PAHs in the urban environment. This paper reviews the literature on PAHs in conjunction with particulate matter on a global perspective.

Effect of air pollution on preterm birth among children born in Southern California between 1989 and 1993.

Ritz B., Yu F., Chapa G. and Fruin S.

Epidemiology. 2000 11(5):502-11.

We evaluated the effect of air pollution exposure during pregnancy on the occurrence of preterm birth in a cohort of 97,518 neonates born in Southern California. We used measurements of carbon monoxide (CO), nitrogen dioxide, ozone, and particulate matter less than 10 microm (PM10) collected at 17 air-quality-monitoring stations to create average exposure estimates for periods of pregnancy. We calculated crude and adjusted risk ratios (RRs) for premature birth by period-specific ambient pollution levels. We observed a 20% increase in preterm birth per 50-microg increase in ambient PM10 levels averaged over 6 weeks before birth [RR_{crude} = 1.20; 95% confidence interval (CI) = 1.09-1.33] and a 16% increase when averaging over the first month of pregnancy (RR_{crude} = 1.16; 95% CI = 1.06-1.26). PM10 effects showed no regional pattern. CO exposure 6 weeks before birth consistently exhibited an effect only for the inland regions (RR_{crude} = 1.13; 95% CI = 1.08-1.18 per 3 parts per million), and during the first month of pregnancy, the effect was weak for all stations (RR_{crude} = 1.04; 95% CI = 1.01-1.09 per 3 parts per million). Exposure to increased levels of ambient PM10 and possibly CO during pregnancy may contribute to the occurrence of preterm births in Southern California.

Fetal growth and maternal exposure to particulate matter during pregnancy.

Dejmek J., Selevan S. G., Benes I., Solansky I. and Sram R. J.

Environmental Health Perspectives. 1999 107(6):475-80.

Prior studies reported an association between ambient air concentrations of total suspended particles and SO₂ during pregnancy and adverse pregnancy outcomes. We examined the possible impact of particulate matter up to 10 microm (PM10) and up to 2.5 microm (PM_{2.5}) in size on

intrauterine growth retardation (IUGR) risk in a highly polluted area of Northern Bohemia (Teplice District). The study group includes all singleton full-term births of European origin over a 2-year period in the Teplice District. Information on reproductive history, health, and lifestyle was obtained from maternal questionnaires. The mean concentrations of pollutants for each month of gestation were calculated using continuous monitoring data. Three intervals (low, medium, and high) were constructed for each pollutant (tertiles). Odds ratios (ORs) for IUGR for PM10 and PM2.5 levels were generated using logistic regression for each month of gestation after adjustment for potential confounding factors. Adjusted ORs for IUGR related to ambient PM10 levels in the first gestational month increased along the concentration intervals: medium 1.62 [95% confidence interval (CI), 1.07-2.46], high 2.64 (CI, 1.48-4.71). ORs for PM2.5 were 1.26 (CI, 0.81-1.95) and 2.11 (CI, 1.20-3.70), respectively. No other associations of IUGR risk with particulate matter were found. Influence of particles or other associated air pollutants on fetal growth in early gestation is one of several possible explanations of these results. Timing of this effect is compatible with a current hypothesis of IUGR pathogenesis. Seasonal factors, one of the other possible explanations, is less probable. More investigation is required to examine these findings and alternative explanations.

The effect of ambient carbon monoxide on low birth weight among children born in southern California between 1989 and 1993.

Ritz B. and Yu F.

Environmental Health Perspectives. 1999 107(1):17-25.

We evaluated the effect of carbon monoxide (CO) exposures during the last trimester of pregnancy on the frequency of low birth weight among neonates born 1989-1993 to women living in the Los Angeles, California, area. Using birth certificate data for that period, we assembled a retrospective cohort of infants whose mothers resided within 2 miles of 1 of 18 CO monitoring stations. Based on the gestational age and birth date of each child, we estimated last-trimester exposure by averaging the corresponding 3 months of daily CO concentrations registered at the monitoring station closest to the mother's residence (determined from the birth certificate). Where data were available (at 6 stations), we also averaged measurements taken daily for nitrogen dioxide and ozone and those taken at 6-day intervals for particulate matter [less than/equal to]10 microm (PM10) to approximate last-trimester exposures to other pollutants. Overall, the study cohort consisted of 125,573 singleton children, excluding infants born before 37 or after 44 weeks of gestation, those weighing below 1,000 or above 5,500 g at birth, those for whom fewer than 10 days of CO measurements were available during the last trimester, and those whose mothers suffered from hypertension, diabetes, or uterine bleeding during pregnancy. Within the cohort, 2,813 (2.2%) were low in birth weight (between 1,000 and 2,499 g). Exposure to higher levels of ambient CO (>5.5 ppm 3-month average) during the last trimester was associated with a significantly increased risk for low birth weight [odds ratio (OR) = 1.22; 95% confidence interval (CI), 1.03-1.44] after adjustment for potential confounders, including commuting habits in the monitoring area, sex of the child, level of prenatal care, and age, ethnicity, and education of the mother.

Association between air pollution and intrauterine mortality in Sao Paulo, Brazil.

Pereira L. A., Loomis D., Conceicao G. M., Braga A. L., Arcas R. M., Kishi H. S., Singer J. M., Bohm G. M. and Saldiva P. H.

Environmental Health Perspectives. 1998 106(6):325-9.

The associations among daily counts of intrauterine mortality and pollutant concentrations (NO₂, SO₂, CO, O₃, and particulate matter (3/4)10 microm) were investigated for the period ranging from January 1991 to December 1992 in the city of Sao Paulo, Brazil. We used Poisson regression techniques, adjusted for season and weather. The association between intrauterine mortality and air pollution was strong for NO₂ (coefficient = 0.0013/ microg/m³; p<0.01) but lesser for SO₂ (coefficient = 0.0005/ microg/m³; p<0.10) and CO (coefficient = 0.0223/ppm; p<0.10). A significant association was observed when an index that combined these three pollutants was considered in the models instead of considering each pollutant individually (p<0.01). These associations exhibited a short time lag, not over 5 days. In addition, some evidence of fetal exposure to air pollution was obtained by disclosing a significant association between the levels of carboxyhemoglobin of blood sampled from the umbilical cord and ambient CO levels in children delivered by nonsmoking pregnant women in the period from May to July 1995. Our results suggest that air pollution in Sao Paulo may promote adverse health effects on fetuses.

B. Meeting abstracts reporting increased risk of adverse developmental or reproductive outcomes

A time series analysis of air pollution and preterm birth in Pennsylvania, 1997-2001.

Herring A., Neas L., Savitz D., Poole C., Sagiv S., Mendola P. and Loomis D.
Epidemiology 2004;15(4).

Introduction: Small increases in risk for preterm delivery in relation to air pollution have been reported, but prior investigations may have inadequately controlled for individual factors, such as maternal smoking. To eliminate confounding by known and unknown individual risk factors that do not vary over short periods of time, we conducted a times-series analysis to investigate the effect of ambient outdoor particulate matter with diameter of 10 micrometers or less (PM10) and sulfur dioxide (SO2) on risk for preterm delivery. Methods: Daily counts of preterm births were obtained from birth records in four Pennsylvania counties (Allegheny, Beaver, Lackawanna and Philadelphia) between January 1, 1997 and December 31, 2001. PM10 and SO2 levels were averaged over a six-week window directly preceding birth. Full, adjusted models controlled for long-term seasonal trends, co-pollutants (nitrogen dioxide, carbon monoxide and SO2 in the PM10 analysis/PM10 in the SO2 analysis), and were offset by the number of live births in the population. Long-term seasonal trends were adjusted using county-specific parametric splines and county-level information was incorporated using a mixed model with a random intercept. Results: Of 187,997 singleton births, 21,450 (11.4%) were born preterm. Mean six-week air pollution for the four counties ranged from 8.67 to 68.85 ug/m³ for PM10 and from 0.79 to 17.02 parts per billion (ppb) for SO2. In full, adjusted models, we observed approximately 1 excess preterm birth for every 100 births exposed to a 50 ug/m³ increase in PM10 (Risk Ratio=1.09; 95% CI: 1.01, 1.19) and 2 excess preterm birth for every 100 births exposed to a 15 ppb SO2 increase (RR =1.20%, 95% CI: 1.04, 1.39). Discussion: These findings provide evidence of small absolute increases in preterm birth risk with exposure to both PM10 and SO2 in the six weeks preceding birth. Results from this time-series analysis of air pollution and preterm delivery are consistent with prior investigations using conventional analyses. This suggests that confounding at the individual level is probably not explaining the observed association between air pollution and preterm delivery. Further analyses are planned to disentangle the effects of PM10 and SO2 and to investigate a possible acute effect of these pollutants in the seven days preceding birth.

Investigating adverse birth outcomes and air pollution in Michigan.

Sadeghnejad A., Hultin M. L., Batterman S., Depa M., Wirth J. and Wahl R. L.
Epidemiology 2004;15(4).

Introduction: A growing body of literature indicates that exposure to ambient levels of air pollutants adversely affects fetal development. Exposure to the criteria air pollutants carbon monoxide (CO), nitrogen dioxide (NO2), ozone (O3), particulate matter less than 2.5 m (PM2.5) and sulfur dioxide (SO2) have been associated with low birth weight, premature birth and small for gestational aged neonates. To assess the potential effects of these pollutants, a semi-individual study was designed to merge databases containing levels of criteria air pollutants

measured at four air monitors in two areas of Michigan with a database containing individual level birth certificate information. Methods: Birth certificate data from 1990-2001 was obtained on births among residents of 36 zip codes in Allen Park (AP), Lansing (LA) and in East Seven Mile (E7) and Linwood (LI) in Detroit. Zip codes within a 4- kilometer radius of an air monitoring station providing data on levels of CO, NO₂, O₃, PM_{2.5}, SO₂, and meteorological assessments were included. Among infants of 16-45 year old mothers, singletons weighing 750-4000g with a gestational age of 20-40 weeks were included. The primary adverse birth outcome is low birth weight (LBW, < 1500g). LBW risk factors in the study included maternal race, age, prenatal care level (Kessner Index), site of residence, history of certain diseases, and smoking, as well as gender of the newborn and year and month of the delivery. Results: Analyses included 207,481 newborns (80% of the initial sample). The ratio of African-American/Caucasian and mean birth weight was 6/94 and 3245g in AP, 76/24 and 3048g in LI, 65/35 and 3094g in E7, and 20/80 and 3212g in LA, respectively. Mean gestational age was approximately 37 weeks at all sites. Risk factors (OR and 95% CI > 1.0) for LBW were low gestational age (< 38 weeks), maternal diseases (except for diabetes), maternal smoking, African-American ethnicity of the mother, female newborn, age of the mother (< 20 years and > 30 years) and the site of residence (others versus AP). Discussion: In accordance with previous studies, the analysis indicated that low birth weight is partially explained by maternal health conditions, habits, and ethnicity as well as newborn's gestational age and gender. Several regression models for each pollutant as the exposure variable and controlling for LBW risk factors are being examined. In the final model, we will include all pollutants that show a significant effect in singlepollutant models.

Air pollution and risk for preterm birth in Pennsylvania, 1997-2001: A time series approach.

Sagiv S., Mendola P., Loomis D., Herring A., Neas L., Savitz D. and Poole C.
American Journal of Epidemiology. 2004;159(11 Suppl).

Preterm delivery can lead to serious infant health outcomes including death and life-long disability. Small increases in preterm delivery risk in relation to air pollution have been reported, but prior investigations may have inadequately controlled for individual factors, such as smoking. We conducted a time-series analysis, which eliminates confounding by individual factors, to investigate the effect of ambient outdoor particulate matter with diameter of ≤ 10 micrometers (PM₁₀) and sulfur dioxide (SO₂) on risk for preterm delivery. Daily counts of preterm births were obtained from birth records in four Pennsylvania counties between 1997 and 2001. PM₁₀ and SO₂ levels were averaged over a six-week window directly preceding birth. Full, adjusted models controlled for long-term seasonal trends, co-pollutants (nitrogen dioxide, carbon monoxide, SO₂, PM₁₀), and were offset by the number of live births in the population. County-level information was incorporated using a mixed model with a random intercept. Of 187,997 singleton births, 21,450 (11.4%) were born preterm. In full, adjusted models, we observed approximately 1 excess preterm birth for every 100 births exposed to a 50 $\mu\text{g}/\text{m}^3$ increase in PM₁₀ (Risk Ratio = 1.09; 95% CI: 1.01, 1.19) and 2 excess preterm births for every 100 births exposed to a 15 parts per billion (ppb) SO₂ increase (RR = 1.20%, 95% CI: 1.04, 1.39). While the absolute increases in risk observed here are small, they may have important public health impact due to the high prevalence of preterm birth.

Ambient air pollution and risk of birth defects in Southern California.

Ritz B. R., Yu F. and Shaw G.

American Journal of Epidemiology. 2001;153(11).

We evaluated the effect of air pollution on the occurrence of birth defects ascertained by the California Birth Defect Monitoring Program in Southern California between 1987 and 1993. Using measurements of carbon monoxide (CO), nitrogen dioxide (NO₂), ozone (O₃) and particulate matter less than 10 micron in aerodynamic diameter (PM₁₀) collected at 30 ambient air-quality monitoring stations we created average monthly exposure estimates for the first trimester of pregnancy. We calculated odds ratios (OR) for subgroups of cardiac and orofacial defects and employed hierarchical and polytomous logistic regression to adjust for multiple comparisons. Odds ratios for cardiac ventricular septal defect (VSD) increased with CO exposure in a dose-response fashion during the second month of pregnancy (OR 2nd quartile CO = 1.62, 95% confidence interval (CI) 1.05-2.48; OR 3rd quartile CO = 2.09, 95% CI 1.19-3.67; OR 4th quartile CO = 2.95, 95% CI 1.44-6.05). We also observed an increased risk for aortic artery and valve defects, pulmonary artery and valve anomalies, and conotruncal defects with increasing second month ozone exposure of similar size. Our results suggest that in Southern California second month CO and Ozone exposure may contribute to the occurrence of VSDs, aortic artery and valve defects, and possibly pulmonary artery and valve anomalies and conotruncal defects. These results are supported by the specificity of the timing of the effect and some evidence from animal data, but since this is the first study to ever link ambient air pollution to human malformations confirmation of these effects by other studies is needed.

C. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Low levels of ambient air pollution during pregnancy and fetal growth among term neonates in Brisbane, Australia.

Hansen C., Neller A., Williams G. and Simpson R.

Environmental Research. 2007 103(3):383-9.

There is mounting evidence that maternal exposure to ambient air pollution during pregnancy is associated with adverse birth outcomes. We examined birth weight and small for gestational age (SGA <10th percentile for age and gender) among 26,617 singleton full-term births in Brisbane, Australia (July 2000-June 2003), in relation to ambient pollution during pregnancy. We also examined head circumference (HC) and crown-heel length (CHL) among a sub-sample (n=21,432) of the term neonates. Maternal exposure to PM₁₀, visibility reducing particles (bsp), O₃ and NO₂ was assessed by calculating average exposure estimates over months and trimesters of pregnancy based on a citywide average of the pollutants. Linear and logistic regression models were employed to examine the effect of these pollutants on the birth outcomes

after adjusting for potential confounders and season of birth. The regression coefficients were based on an inter-quartile range (IQR) increase in exposure as well as quartiles of exposure with the lowest used as a reference category. Trimester- and monthly specific exposures to all pollutants were not significantly associated with a reduction in either birth weight or HC, or an increased risk of SGA. An IQR increase in NO₂ during the third trimester was associated with a reduction in CHL (beta=-0.15cm, 95% CI -0.25 to -0.05cm) and this was concentrated around exposure during month nine. No other pollutants were associated with a reduction in CHL. In conclusion, there was no strong evidence suggesting that ambient air pollution during pregnancy is associated with sub-optimal fetal growth in Brisbane.

Effects of ambient air pollution on gestational age, Sydney, Australia.

Jalaludin B., Morgan G., Lincoln D., Sheppard V., Corbett S. F. and Mannes T.
Epidemiology 2003;14(5 Suppl). (Meeting Abstract)

Introduction: There are no reported studies on the effects of ambient air pollution on birth outcomes from Australia. We therefore undertook an investigation to determine if there were any associations between ambient air pollutants (particulate matter, nitrogen dioxide and ozone) and gestation. The study was conducted in Sydney, Australia. Methods: The study period was a three-year period between 1998 and 2000 and included all births in metropolitan Sydney in this time period. We used routinely collected data for both the exposures and birth outcomes. We obtained the perinatal data from the New South Wales Health Department and air pollution data from the New South Wales Environment Protection Authority. Birth data included demographic factors (age, smoking status, country of birth, postcode of residence at time of delivery), pregnancy factors (date of the last menstrual period, gestational hypertension and diabetes, parity, time of first antenatal visit to a healthcare provider), details about the delivery (type of delivery) and infant factors (birth weight, Apgar score). Air pollution data included information on particulate matter (PM₁₀, PM_{2.5}; 24-hour averages), nitrogen dioxide (NO₂; 1-hour average), ozone (O₃; 1-hour average) and carbon monoxide (CO; 1-hour average). Exposure periods were defined as 30 days and 90 days prior to delivery and 30 days and 90 days after the last menstrual period. Exposures were averaged across all air monitoring Stations in metropolitan Sydney. Gestation was analysed as a categorical Variable (< 37 weeks and > 37 weeks) using logistic regression. Results: Over the three year period there were 122,877 singleton births to mothers who did not have gestational hypertension and diabetes. 4.7% (n = 5831) of these births were preterm (< 37 weeks at birth). Median air pollutant concentrations were: PM₁₀ 24-h average 15.7mg/m³, PM_{2.5} 24-h average = 8.4mg/m³, NO₂ 1-h maximum 23.0 ppb, O₃ 1-h maximum = 27.7 ppb and CO 1-h maximum = 1.2 ppm. There was no effect of particulates or gaseous pollutants averaged over the month or three months prior to delivery on gestation. Particulates, NO₂ and CO exposure in the first one and three months of pregnancy was associated with a lower risk for prematurity, whereas ozone exposure in the first three months of pregnancy increased the risk of preterm delivery. Conclusions: Preliminary analyses suggest that in Sydney, Australia we were not able to demonstrate consistent adverse effects of ambient air pollution on preterm delivery. Exposure to ambient ozone in the first trimester was associated with an increased risk of preterm deliveries. However, as this was the only significant adverse finding among the many tests done, we interpret the results.

Effects of ambient air pollution on birth weight in term infants, Sydney, Australia.

Jalaludin B., Morgan G., Lincoln D., Sheppard V., Corbett S. F. and Mannes T.

Epidemiology 2003 14(5 Suppl):S130-S31. (Meeting Abstract)

Introduction: There are no reported studies on the effects of ambient air pollution on birth outcomes from Australia. We therefore undertook a, investigation to determine if there we, any associations between ambient air pollutants (particulate matter, nitrogen dioxide, ozone and carbon monoxide) and birth weight. The study was conducted in Sydney, Australia. Methods: The study period was between 1998 and 2000 and included all births in metropolitan Sydney in this time period. We used routinely collected data for both the exposures and birth outcomes. We obtained the perinatal data from the New South Wales Health Department and air pollution data from the New South Wales Environment Protection Authority. Birth data included maternal demographic factors (age, smoking status, country of birth, postcode of residence at time of delivery), pregnancy factors (date of the last menstrual period, gestational hypertension and diabetes, parity, time of first antenatal visit to a healthcare provider), details about the delivery (type of delivery) and infant factors (birth weight, Apgar score). The air pollution data included information on particulate matter (PM10, PM2.5; 24-hour averages), nitrogen dioxide (NO2; 1-hour average), ozone (O3; 1-hour average) and carbon monoxide (CO; 1-hour average). Exposure periods were defined as 30 days and 90 days prior to delivery and 30 days and 90 days after the last menstrual period. Exposures were averaged across all air monitoring stations in metropolitan Sydney. Birth weight was analysed as a categorical variable (< 2500 grams and > 2500 grams) in logistic regression models. Results: Over the three year period there were 117018 singleton term births (37-42 weeks gestation) to mothers who did not have gestational hypertension and diabetes. 1.5% of these births were low birth weight (less than 2500 grams at birth). Median air pollutant concentrations were: PM10 24-h average = 15.7mg/m³, PM2.5 24-h average = 8.4mg/m³, NO2 1-h maximum = 23.0ppb, O3 1-h maximum = 27.7ppb and CO 1-h maximum = 1.2ppm. Preliminary analyses suggest that particulates or gaseous pollutants exposure in the month or three months prior to delivery were not associated with low birth weight. However, NO2 and CO exposure in the first one and three months of pregnancy was associated with a lower risk for low birth weight. Conclusions: In Sydney, Australia we were not able to demonstrate adverse effects of ambient air pollution on birth weight. Reasons for this may be the low levels of ambient air pollutants in metropolitan Sydney or differences in the ambient air pollution mix.

Community study of spontaneous abortions: relation to occupation and air pollution by sulfur dioxide, hydrogen sulfide, and carbon disulfide.

Hemminki K, Niemi ML.

International Archives of Occupational and Environmental Health. 1982 51(1):55-63.

Spontaneous abortions were analyzed in an industrial community in Finland in relation to the occupation of women and their husbands, and to the level of air pollution in the family's residential area. Information on abortions and births was obtained from the hospital discharge

register; information on the women and their families was obtained from the files of the population and housing census. Women who were employed in rayon textile jobs and paper products jobs had an increased rate (P less than 0.10) of spontaneous abortions; the wives of men employed in transport and communication, in rayon textile jobs, and in chemical process jobs also had an increased rate of spontaneous abortions. In material stratified for age, parity, and socioeconomic class no evidence was found that the level of sulfur dioxide or carbon disulfide could be associated with a risk of spontaneous abortions. More spontaneous abortions were noted in all socioeconomic classes in areas where the mean annual level of hydrogen sulfide exceeded 4 micrograms m⁻³. However, the difference (total rates 7.6 and 9.3, respectively) was not significant statistically.

D. Related articles

Meeting report: atmospheric pollution and human reproduction.

Slama R., Darrow L., Parker J., Woodruff T. J., Strickland M., Nieuwenhuijsen M., Glinianaia S., Hoggatt K. J., Kannan S., Hurley F., Kalinka J., Sram R., Brauer M., Wilhelm M., Heinrich J. and Ritz B.

Environmental Health Perspectives. 2008 116(6):791-8.

BACKGROUND: There is a growing body of epidemiologic literature reporting associations between atmospheric pollutants and reproductive outcomes, particularly birth weight and gestational duration. **OBJECTIVES:** The objectives of our international workshop were to discuss the current evidence, to identify the strengths and weaknesses of published epidemiologic studies, and to suggest future directions for research. **DISCUSSION:** Participants identified promising exposure assessment tools, including exposure models with fine spatial and temporal resolution that take into account time-activity patterns. More knowledge on factors correlated with exposure to air pollution, such as other environmental pollutants with similar temporal variations, and assessment of nutritional factors possibly influencing birth outcomes would help evaluate importance of residual confounding. Participants proposed a list of points to report in future publications on this topic to facilitate research syntheses. Nested case-control studies analyzed using two-phase statistical techniques and development of cohorts with extensive information on pregnancy behaviors and biological samples are promising study designs. Issues related to the identification of critical exposure windows and potential biological mechanisms through which air pollutants may lead to intrauterine growth restriction and premature birth were reviewed. **CONCLUSIONS:** To make progress, this research field needs input from toxicology, exposure assessment, and clinical research, especially to aid in the identification and exposure assessment of fetotoxic agents in ambient air, in the development of early markers of adverse reproductive outcomes, and of relevant biological pathways. In particular, additional research using animal models would help better delineate the biological mechanisms underpinning the associations reported in human studies.

[Air pollutant exposure during pregnancy and fetal and early childhood development. Research protocol of the INMA (Childhood and Environment Project)].

Esplugues A., Fernandez-Patier R., Aguilera I., Iniguez C., Garcia Dos Santos S., Aguirre Alfaro A., Lacasana M., Estarlich M., Grimalt J. O., Fernandez M., Rebagliato M., Sala M., Tardon A., Torrent M., Martinez M. D., Ribas-Fito N., Sunyer J. and Ballester F.
Gaceta sanitaria / SESPA. 2007 21(2):162-71.

INTRODUCTION: The INMA (Infancia y Medio Ambiente [Spanish for Environment and Childhood]) project is a cooperative research network. This project aims to study the effects of environment and diet on fetal and early childhood development. This article aims to present the air pollutant exposure protocol during pregnancy and fetal and early childhood development of the INMA project. **METHODS:** The information to assess air pollutant exposure during pregnancy is based on outdoor measurement of air pollutants (nitrogen dioxide [NO₂], volatile organic compounds [VOC], ozone, particulate matter [PM₁₀, PM_{2,5}] and of their composition [polycyclic aromatic hydrocarbons]); measurement of indoor and personal exposure (VOC and NO₂); urinary measurement of a biological marker of hydrocarbon exposure (1-hydroxypyrene); and data gathered by questionnaires and geographic information systems. These data allow individual air pollutant exposure indexes to be developed, which can then be used to analyze the possible effects of exposure on fetal development and child health. **CONCLUSION:** This protocol and the type of study allow an approximation to individual air pollutant exposure to be obtained. Finally, the large number of participants (N = 4,000), as well as their geographic and social diversity, increases the study's potential.

Environmental factors and developmental outcomes in the lung.

Kajekar R.
Pharmacology and Therapeutics. 2007 114(2):129-45.

The developing lung is highly susceptible to damage from exposure to environmental toxicants particularly due to the protracted maturation of the respiratory system, extending from the embryonic phase of development in utero through to adolescence. The functional organization of the lungs requires a coordinated ontogeny of critical developmental processes that include branching morphogenesis, cellular differentiation and proliferation, alveolarization, and maturation of the pulmonary immune, vasculature, and neural systems. Therefore, exposure to environmental pollutants during crucial periods of prenatal and/or postnatal development may determine the course of lung morphogenesis and maturation. Depending on the timing of exposure and pathobiological response of the affected tissue, exposure to environmental pollutants can potentially result in long-term alterations that affect the structure and function of the respiratory system. Besides an immature respiratory system at birth, children possess unique differences in their physiology and behavioral characteristics compared to adults that are believed to augment the vulnerability of their developing lungs to perturbations by environmental toxins. Furthermore, an interaction between genetic predisposition and increased opportunity for exposure to chemical and infectious disease increase the hazards and risks for infants and children. In this article, the evidence for perturbations of lung developmental processes by key ambient pollutants (environmental tobacco smoke [ETS], ozone, and

particulate matter [PM]) are discussed in terms of biological factors that are intrinsic to infants and children and that influence exposure-related lung development and respiratory outcomes.

PM2.5 and PM10 induce the expression of adhesion molecules and the adhesion of monocytic cells to human umbilical vein endothelial cells.

Montiel-Davalos A., Alfaro-Moreno E. and Lopez-Marure R.
Inhalation Toxicology. 2007 19 Suppl 1:91-8.

Exposure to airborne particles has been associated with an increase in cardiopulmonary events. Endothelial cells could be playing an important role in the response to airborne particles due their involvement in proinflammatory events, and there is some evidence of particle translocation from lung into circulation. One of the initiating events of inflammation is endothelial activation. We determined the concentration-response effect of a particulate matter with different aerodynamic sizes (PM2.5 [particulate matter with aerodynamic diameter of 2.5 microm and less] and PM10 [particulate matter with aerodynamic diameter of 10 microm and less]) obtained from Mexico City on human umbilical vein endothelial cells (HUVEC). The adhesion of monocytic U937 cells to HUVEC and the expression of early (E- and P-selectins) and late (ICAM-1, PECAM-1, VCAM-1) adhesion molecules were tested. Adhesion of U937 cells to HUVEC was evaluated by coculture experiments using [3H]thymidine-labeled U937 cells and the expression of adhesion molecules was evaluated by flow cytometry. Tumor necrosis factor (TNF)-alpha was used as a positive control of endothelial activation. Our results showed that both PM2.5 and PM10 induced the adhesion of U937 cells to HUVEC, and their maximal effect was observed at 20 microg/cm2. This adhesion was associated with an increase in the expression of all adhesion molecules evaluated for PM10, and E-selectin, P-selectin, and ICAM-1 for PM2.5. In general, maximum expression of adhesion molecules induced by PM2.5 and PM10 was obtained with 20 microg/cm2; however, PM10-induced expression was observed from 5 microg/cm2. E-selectin and ICAM-1 had the strongest expression in response to particles. In conclusion, PM2.5 and PM10 induce the activation of HUVEC, leading to monocytic adhesion via the expression of adhesion molecules, suggesting that these particles may participate in the development of inflammatory diseases. The role of these events in the development of diseases such as atherosclerosis is likely to be evaluated.

Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition.

Kannan S., Misra D. P., Dvonch J. T. and Krishnakumar A.
Environmental Health Perspectives. 2006 114(11):1636-42.

OBJECTIVES: The specific objectives are threefold: to describe the biologically plausible mechanistic pathways by which exposure to particulate matter (PM) may lead to the adverse perinatal outcomes of low birth weight (LBW), intrauterine growth retardation (IUGR), and preterm delivery (PTD); review the evidence showing that nutrition affects the biologic pathways; and explain the mechanisms by which nutrition may modify the impact of PM exposure on perinatal outcomes. **METHODS:** We propose an interdisciplinary conceptual framework that brings together maternal and infant nutrition, air pollution exposure assessment,

and cardiopulmonary and perinatal epidemiology. Five possible albeit not exclusive biologic mechanisms have been put forth in the emerging environmental sciences literature and provide corollaries for the proposed framework. **CONCLUSIONS:** Protecting the environmental health of mothers and infants remains a top global priority. The existing literature indicates that the effects of PM on LBW, PTD, and IUGR may manifest through the cardiovascular mechanisms of oxidative stress, inflammation, coagulation, endothelial function, and hemodynamic responses. PM exposure studies relating mechanistic pathways to perinatal outcomes should consider the likelihood that biologic responses and adverse birth outcomes may be derived from both PM and non-PM sources (e.g., nutrition). In the concluding section, we present strategies for empirically testing the proposed model and developing future research efforts.

Perceived maternal psychosocial stress in patients exposed to varying levels of particulate matter (PM) and air pollution: Is there an association? A crosssectional pilot.

Rouse H. A. R., Gilliland F., McConnell R., Jerrett M. and McGregor J. A.

Journal of the Society for Gynecologic Investigation. 2006 13(2):226A-27A.

BACKGROUND: Particulate matter (PM), a component of air pollution derived from diesel exhaust, may negatively impact pregnancy outcomes. Studies of nonpregnant Los Angeles County residents demonstrate associations between outdoor PM levels and adverse health outcomes. Growing data illustrate the role of perceived psychosocial stress as a modifier of health and systemic disease. Circumstances surrounding women who live in highly polluted environments also likely produce significant psychosocial stress. **HYPOTHESIS:** Pregnant women who live in areas that have high levels of air pollution perceive moderate to high levels of psychosocial stress. **METHODS:** Women who presented for delivery of a term singleton at L.A. County Women's and Children's Hospital were prospectively enrolled. A survey was implemented to collect epidemiologic data, PM exposure assessment, perceived level of personal stress, and pregnancy outcome data. Survey data estimated PM exposure by patient-reported distance from primary residence to freeway or major thoroughfare. PM exposure was designated as 'high' if subjects lived within 500 m, or 3 city blocks, from a heavily trafficked road. Other pollution exposure, such as smoking, was also assessed. Exposure data were correlated with demographic data and scored responses to survey questions regarding personal stress. **RESULTS:** Data were analyzed for patients whose records were complete (n=19). Median age was 27 years (range 18-40), and median gestational age was 39 weeks (range 37-41 weeks). The majority of subjects were Latina (n=16). Eighty-eight percent of subjects reported 'high' air pollution exposure levels. Median birth weight was 3467.5 g (range 2790- 4295 g). All subjects who live > 500 m from a heavily trafficked street reported low stress scores (n=2). All subjects who described living immediately next to a busy street reported stress scores in the moderate or high range (n=3). Of subjects living < 1 city block from a heavily trafficked street, 50% reported moderate stress scores and 50% reported low stress scores (n=4). Of subjects living between 1-3 blocks from a busy street, 66% scored in the low stress range (n=4). **CONCLUSION:** These pilot data suggest an increased risk of high levels of perceived stress in patients exposed to 'high' levels of PM. Further study is needed to define a relationship between PM exposure and perception of stress, and between these factors and pregnancy outcomes.

Residential environmental risks for reproductive age women in developing countries.

Dyjack D., Soret S., Chen L., Hwang R., Nazari N. and Gaede D.

Journal of Midwifery & Women's Health. 2005 50(4):309-14.

Published research suggests there is an association between maternal inhalation of common ambient air pollutants and adverse birth outcomes, including an increased risk for preterm delivery, intrauterine growth retardation, small head circumference, low birth weight, and increased rate of malformations. The air pollutants produced by indoor combustion of biomass fuels, used by 50% of households worldwide, have been linked to acute lower respiratory infections, the single most important cause of mortality in children under the age of 5. This report describes a hypothesis-generating study in West Wollega, Ethiopia, conducted to assess airborne particulate matter concentrations in homes that combust biomass fuels (biomass homes). Respirable suspended particulate matter was measured in biomass homes and nonbiomass homes using NIOSH method 0600. Measured airborne particulate concentrations in biomass homes were up to 130 times higher than air quality standards. These findings, in part, confirm that exposure to indoor air pollutants are a major source of concern for mother/child health. Midwives are encouraged to raise awareness, contribute to research efforts, and assist in interventions.

NF-kappaB activation in human JEG3 trophoblast cells and murine placental fibroblasts after exposure to ultrafine particulate matter.

Rouse-Ho A., McGregor J., Kleinman M., Lu D., Wilson M., Khoury N., Simmons C. and Equils O.

American Journal of Obstetrics and Gynecology. 2005 193(6):S183.

OBJECTIVE: Air pollution is implicated in causing inflammatory-mediated respiratory and cardiovascular disease, and may contribute to pregnancy disorders. Inhaling small particles from diesel fuel emissions, among other sources, stimulates local inflammatory responses in the lung and has been associated with a systemic proinflammatory state. Particulate matter may cause placental inflammation and increase the risk of preterm birth. The purpose of this study is to demonstrate that particulate matter induces NF-kappaB activation in human JEG3 trophoblast cells and murine placental fibroblasts. **STUDY DESIGN:** JEG3 human trophoblast cells and primary murine placental fibroblast cells were transfected with NF-kappaB luciferase and betagalactosidase expression vectors using Fugene 6 (Boehringer Mannheim, Indianapolis, IN) overnight. The cells were then stimulated with commercially prepared LPS-free particles measuring 0.1 micron (ultrafine) or measuring 0.98 micron (fine) overnight. Control groups were treated with serum-free medium alone or with lipopolysaccharide (LPS). NF-kappaB luciferase activity was assessed by luciferase assay and use of a luminometer. Calorimetric betagalactosidase assay was performed to correct for transfection efficiency. The NF-kappaB activation in the experimental groups was compared to the activity of the media-treated or LPS-stimulated cells. Statistical significance was calculated using ANOVA and student t-test. **RESULTS:** Data analysis suggested that exposure to particulate matter induced NF-kappaB activation in JEG3 trophoblasts after a twenty-four hour stimulation ($p=0.02$), with a trend

toward NF-kappaB activation in cells exposed to ultrafine particles (0.1 micron) ($p=0.14$). Data from placental fibroblasts suggest a trend toward activation of NF-kappaB by ultrafine particles as well ($p=0.079$). Fine particles (0.98 micron) did not induce NF-kappaB activation in either cell type. CONCLUSION: Ultrafine particles may activate NF-kappaB in human trophoblast cells and murine placental fibroblasts, and may contribute to local and systemic inflammation in pregnancy.

Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence.

Glinianaia S. V., Rankin J., Bell R., Pless-Mulloli T. and Howel D.
Epidemiology 2004 15(1):36-45.

BACKGROUND: Research on the potential impact of air pollution on the health of adults and children has grown rapidly over the last decade. Recent studies have suggested that air pollution could also be associated with adverse effects on the developing fetus. This systematic review evaluates the current level of epidemiologic evidence on the association between ambient particulate air pollution and fetal health outcomes. We also suggest further research questions. **METHODS:** Using database searches and other approaches, we identified relevant publications published between 1966 and 2001 in English. Articles were included if they reported original data on birthweight, gestational age at delivery, or stillbirth related to directly measured nonaccidental exposure to particulate matter. **RESULTS:** Twelve studies met the inclusion criteria. There was little consistency in the evidence linking particulate air pollution and fetal outcomes. Many studies had methodologic weaknesses in their design and adjustment for confounding factors. Even in well-designed studies, the reported magnitude of the effects was small and inconsistently associated with exposure at specific stages of pregnancy. **CONCLUSIONS:** The currently available evidence is compatible with either a small adverse effect of particulate air pollution on fetal growth and duration of pregnancy or with no effect. Further research should be directed toward clarifying and quantifying these possible effects and generating testable hypotheses on plausible biologic mechanisms.

Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population.

Perera F. P., Rauh V., Tsai W. Y., Kinney P., Camann D., Barr D., Bernert T., Garfinkel R., Tu Y. H., Diaz D., Dietrich J. and Whyatt R. M.
Environmental Health Perspectives. 2003 111(2):201-5.

Inner-city, minority populations are high-risk groups for adverse birth outcomes and also are more likely to be exposed to environmental contaminants, including environmental tobacco smoke (ETS), polycyclic aromatic hydrocarbons (PAHs), and pesticides. In a sample of 263 nonsmoking African-American and Dominican women, we evaluated the effects on birth outcomes of prenatal exposure to airborne PAHs monitored during pregnancy by personal air sampling, along with ETS estimated by plasma cotinine, and an organophosphate pesticide (OP) estimated by plasma chlorpyrifos (CPF). Plasma CPF was used as a covariate because it was the most often detected in plasma and was highly correlated with other pesticides frequently detected

in plasma. Among African Americans, high prenatal exposure to PAHs was associated with lower birth weight ($p = 0.003$) and smaller head circumference ($p = 0.01$) after adjusting for potential confounders. CPF was associated with decreased birth weight and birth length overall ($p = 0.01$ and $p = 0.003$, respectively) and with lower birth weight among African Americans ($p = 0.04$) and reduced birth length in Dominicans ($p < 0.001$), and was therefore included as a covariate in the model with PAH. After controlling for CPF, relationships between PAHs and birth outcomes were essentially unchanged. In this analysis, PAHs and CPF appear to be significant independent determinants of birth outcomes. Further analyses of pesticides will be carried out. Possible explanations of the failure to find a significant effect of PAHs in the Hispanic subsample are discussed. This study provides evidence that environmental pollutants at levels currently encountered in New York City adversely affect fetal development.

The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome.

Dejmek J., Solansky I., Benes I., Lenicek J. and Sram R. J.
Environmental Health Perspectives. 2000 108(12):1159-64.

The relationship between intrauterine growth retardation (IUGR) and exposure to particulate matter [less than/equal to] 10 microm (PM(10)) and particulate matter [less than and equal to] 2.5 microm (PM(2.5)) in early pregnancy was recently studied in the highly polluted district of Teplice (Northern Bohemia). From this observation rose the question about the possible role of the carcinogenic fraction of polycyclic aromatic hydrocarbons (c-PAHs), which are usually bound to fine particles. The impact of c-PAHs and fine particles on IUGR was analyzed in Teplice and in Prachatice, a region with similarly high c-PAH but low particle levels. All European, single live births occurring in a 4-year period in Teplice ($n = 3,378$) and Prachatice ($n = 1,505$) were included. Detailed personal data were obtained via questionnaires and medical records. Mean PM(10), PM(2.5,) and c-PAHs levels during the 9 gestational months (GM) were estimated for each mother. Adjusted odds ratios (AORs) of IUGR for three levels of c-PAHs (low, medium, and high) and for continuous data were estimated after adjustment for a range of covariates using logistic regression models. In the present 4-year sample from Teplice, previously published results about increasing IUGR risk after exposure to particles in the first GM were fully confirmed, but no such effects were found in Prachatice. The AOR of IUGR for fetuses from Teplice exposed to medium levels of c-PAHs in the first GM was 1.60 [confidence interval (CI), 1.06-2.15], and to high levels 2.15 (CI, 1.27-3.63). An exposure-response relationship was established by analyzing the continuous data. For each 10 ng increase of c-PAHs in the first GM, the AOR was 1.22 (CI, 1.07-1.39). About the same relationship was observed in Prachatice in spite of the low particle levels. The results prove that exposure to c-PAHs in early gestation may influence fetal growth. The particulate matter-IUGR association observed earlier may be at least partly explained by the presence of c-PAHs on particle surfaces.

Issues of human exposure to agents causing developmental toxicity.

Tabacova S. and Vukov M.

Congenital Anomalies. 1992 32(Suppl):S21-S30.

The evaluation of human developmental toxicity is a complex process. Specific examples are based on a study which assessed the relationship of multiple environmental exposures and reproductive outcomes in areas contaminated by industrial sources (e.g. metallurgy, chemical and petrochemical industry). Rates of spontaneous abortions, malformations, stillbirths, prematurity, maternal toxemia, and early neonatal morbidity and mortality were determined for a 6-year retrospective period which included 238,221 births and 30,579 spontaneous abortions. Exposures via ambient air (SO₂, NO₂, H₂S, particulates, lead aerosols), soil and water (e.g. Pb, Cd, Cr, Mn, Zn) were studied in parallel. A multiple regression analysis was used to assess the relationships among environmental and reproductive parameters. Other known developmental hazards also were considered (e.g. background radiation, rubella and varicella morbidity, and pertinent disease states). It was found that the excess of heavy metals (Pb, Cd, Cr, Mn), the deficit of essential elements (Zn, Cu, Ni), and particularly the combination of both, had significant relations to the rate of unfavorable reproductive outcomes. Air pollution by H₂S, NO₂, and particulate matter was found to contribute, although to a lesser extent, to some reproductive and developmental pathologies. Background morbidity of rubella, as well as of renal, cardiovascular and endocrine diseases was also of importance. It is suggested that the interplay of various environmental factors is essential in evaluating their role as developmental hazards.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Diesel exhaust particle toxicity on spermatogenesis in the mouse is aryl hydrocarbon receptor dependent.

Izawa H., Kohara M., Watanabe G., Taya K. and Sagai M.
The Journal of Reproduction and Development. 2007 53(5):1069-78.

Diesel exhaust particles (DEPs) are particulate matter from diesel exhaust containing many toxic compounds, such as polyaromatic hydrocarbons (PAHs). Some toxicities of PAH are considered to express via aryl hydrocarbon receptor (AhR). We hypothesized that the male reproductive toxicity of DEPs may depend on PAHs. BALB/c male mice received 24.7, 74.0 or 220 microg/mouse DEP suspension or vehicle injected into the dorsal subcutaneous layer 10 times during 5 weeks. The mice were euthanized, and blood and organs were collected 2 weeks after the last treatment. The epididymis weights, relative epididymis weights per body weight and daily sperm productions and viabilities of the 74.0 and 220 microg/mouse DEP-treated groups decreased significantly compared with those of the vehicle group. The total incidence of sperm abnormalities in the 74.0 and 220 microg/mouse DEP-treated groups increased significantly compared with the vehicle group. The seminiferous epithelium area ratios of the 74.0 and 220 microg/mouse DEP-treated groups were significantly higher compared with the vehicle and 24.6 microg/mouse DEP-treated groups. The ratios of seminiferous tubules with elongated-type spermatids in the 74.0 and 220 microg/mouse DEP-treated groups were significantly decreased compared with the vehicle group. The testosterone level and hepatic ethoxyresorufin-O-deethylase (EROD) activity as an indirect index of AhR activity in the 74.0 microg/mouse DEP-treated group were significantly increased compared with those of the vehicle group. These results clearly demonstrated that DEPs suppress testicular function, especially spermatogenesis and sperm motility. These effects may be AhR dependent.

Decreased number of sperms and Sertoli cells in mature rats exposed to diesel exhaust as fetuses.

Watanabe N.
Toxicology Letter. 2005 15;155(1):51-8.

This study was conducted to follow up the effects of fetal exposure to diesel exhaust on testicular cell numbers and daily sperm production in adulthood. Thirty-six pregnant rats were divided into five groups: groups exposed to total diesel-engine exhaust containing 1.71 mg/m³ particulate matter and 0.80 ppm nitrogen dioxide (high dose) or 0.17 mg/m³ particulate matter and 0.10 ppm nitrogen dioxide (low dose); groups exposed to filtered exhaust without particles containing 0.80 (high dose) or 0.10 (low dose) ppm nitrogen dioxide; and a group exposed to clean air. Exhaust exposure was performed from gestational day 7 to delivery. The numbers of daily produced sperm, spermatids and Sertoli cells in the diesel-exhaust-exposed groups were

significantly lower than those in the control group on day 96 after birth. The ratio of spermatids/Sertoli cells and the follicle-stimulating hormone levels in the exposed groups were significantly higher. The present study provides evidence for the first time that mature rats exposed to diesel exhaust during fetus show a decrease in the daily production of sperm due to an insufficient number of Sertoli cells. As both the exhaust-exposed groups showed almost the same reactions toward the inhalation, the gaseous phase must have included the responsible toxicants.

[Study of toxicity on male reproductive system of mice induced by SO₂ inhalation].

Zhang B., Liu C. Y. and Meng Z. Q.
Wei Sheng Yan Jiu. 2005 34(2):167-9.

OBJECTIVE: In this paper, toxicity of SO₂ on male reproductive system of mice was studied. **METHODS:** 40 mice were divided to 4 groups (10/group): a group for control, the other 3 groups for SO₂ inhalation (28, 56, 112mg/m³), 4h/d, 7d. **RESULTS:** The activities of GST and G-6-PD, as well as the content of GSH decreased significantly with SO₂ increased. The content of MDA increased evidently with SO₂ increased. **CONCLUSION:** SO₂ can influence GSH oxidation-deoxidation system and cause DNA damage in male reproductive system of mice.

In utero and lactation exposure of rats to 1R4F reference cigarette mainstream smoke: effect on prenatal and postnatal development.

Gaworski C. L., Carmines E. L., Faqi A. S. and Rajendran N.
Toxicological Sciences. 2004 79(1):157-69.

Childhood cognitive and behavioral deficits have been reported in children born to mothers who smoked during pregnancy (Institute of Medicine, 2001). To investigate these potential responses in an animal model, reproductive and neurotoxicity evaluations based on the U.S. FDA guidelines were used to examine the offspring of male and female Sprague-Dawley rats exposed 2 h/day, 7 days/week by nose-only inhalation to whole mainstream smoke total particulate matter (TPM). Concentrations of 150, 300, or 600 mg/m³ were used (males: 4 weeks prior to and during mating; and females: 2 weeks prior to mating, during mating, and through weaning at postnatal day 21). Sham air controls receiving filtered air and cage controls were also maintained. F(1) rats were weighed, identified by gender, examined for clinical signs of toxicity, and evaluated for neurobehavioral effects through postnatal day 65. Parental exposure was evidenced by smoke concentration-related increases in blood carboxyhemoglobin, nicotine, and cotinine and by characteristic cigarette smoke-related rodent respiratory tract histopathology. Also, nicotine and cotinine were found in F(1) blood through the lactation period. Maternal toxicity occurred at concentrations of 300 and 600 mg TPM/m³, where total body weight gain during gestation was significantly ($p < \text{or} = 0.05$) decreased compared to sham controls. While smoke concentration-related decreases in F(1) birth weight and growth were evident (600 mg TPM/m³, significantly different from sham at all time points), no adverse effects on developmental landmarks, including age at vaginal patency or preputial separation, motor activity, acoustic startle response or learning, and memory, were observed in the F(1) generation. This study confirmed that maternal exposure to high levels of mainstream cigarette smoke during

gestation and lactation reduces birth weight and retards growth in the rat neonate; however, the developmental and neurobehavioral testing methodologies employed did not appear to be sensitive for an evaluation of neonatal behavioral effects following parental smoke exposure.

Reduced lung cell proliferation following short-term exposure to ultrafine soot and iron particles in neonatal rats: key to impaired lung growth?

Pinkerton K. E., Zhou Y. M., Teague S. V., Peake J. L., Walther R. C., Kennedy I. M., Leppert V. J. and Aust A. E.

Inhalation Toxicology. 2004 16 Suppl 1:73-81.

Particulate matter (PM) has been associated with a variety of negative health outcomes in children involving the respiratory system and early development. However, the precise mechanisms to explain how exposure to airborne particles may cause adverse effects in children are unknown. To study their influence on early postnatal development, a simple, laminar diffusion flame was used to generate an aerosol of soot and iron particles in the size range of 10 to 50 nm. Exposure of 10-day-old rat pups to soot and iron particles was for 6 h/day for 3 days. The lungs were examined following a single injection of bromodeoxyuridine (BrdU) 2 h prior to necropsy. Neonatal rats exposed to these particles demonstrated no effect on the rate of cell proliferation within terminal bronchioles or the general lung parenchyma. In contrast, within those regions arising immediately beyond the terminal bronchioles (defined as the proximal alveolar region), the rate of cell proliferation was significantly reduced compared with filtered air controls. These findings strongly suggest exposure to airborne particles during early neonatal life has significant direct effects on lung growth by altering cell division within critical sites of the respiratory tract during periods of rapid postnatal development. Such effects may result in altered growth in the respiratory system that may be associated with lifelong consequences.

Reduction of particulate air pollution lowers the risk of heritable mutations in mice.

Somers C. M., McCarry B. E., Malek F. and Quinn J. S.

Science. 2004 304(5673):1008-10.

Urban and industrial air pollution can cause elevated heritable mutation rates in birds and rodents. The relative importance of airborne particulate matter versus gas-phase substances in causing these genetic effects under ambient conditions has been unclear. Here we show that high-efficiency particulate-air (HEPA) filtration of ambient air significantly reduced heritable mutation rates at repetitive DNA loci in laboratory mice housed outdoors near a major highway and two integrated steel mills. These findings implicate exposure to airborne particulate matter as a principal factor contributing to elevated mutation rates in sentinel mice and add to accumulating evidence that air pollution may pose genetic risks to humans and wildlife.

Effect of Diesel Exhaust on Development of Fetal Reproductive Function in ICR Female Mice.

Tsukue N., Yoshida S., Sugawara I. and Taked K.

Journal of Health Science. 2004 50(2):174-80.

Diesel exhaust (DE) is a serious air pollution problem in big cities. Most suspended particulate matter (SPM) less than 2.5 μm in diameter consists of diesel exhaust particles (DEPs), which are reported to cause pulmonary carcinogenesis, allergic rhinitis, and bronchial asthma-like diseases. It has been recently reported that DE also affects the circulatory and reproductive systems. Yoshida et al. reported that mRNA expression of steroidogenic factor-1 (Ad4BP/SF-1) and of Müllerian inhibitory substance (MIS), which are essential for male gonadal differentiation, decreased significantly in male fetuses when maternal mice were exposed to DE at levels of 0.1 or 3.0 mg DEP/ m^3 for 8 hr per day between 2 and 13 days postcoitum (dpc). In this study, maternal mice were exposed to DE 0.1 mg DEP/ m^3 for 8 hr per day between 2 and 13 dpc. Expression levels of Ad4BP/SF-1 and MIS mRNA in female fetuses were not decreased. However, expression levels of bone morphogenetic protein-15, reported to be related to development of the oocyte, were significantly decreased in comparison with that in the control group. Our data suggest that female fetuses of pregnant mice exposed to DE in utero are less sensitive to the expression levels of mRNAs for Ad4BP/SF-1 and MIS compared with males and that DE may affect development of the oocyte in the female fetus.

The masculinization of the fetus during pregnancy due to inhalation of diesel exhaust.

Watanabe N. and Kurita M.

Environmental Health Perspectives. 2001 109(2):111-9.

This study was conducted to determine the impact of diesel exhaust inhalation on the fetus. Seventy-two pregnant rats and 18 nonpregnant rats were divided into three groups: a group exposed to total diesel engine exhaust containing 5.63 mg/ m^3 particulate matter, 4.10 ppm nitrogen dioxide, and 8.10 ppm nitrogen oxide; a group exposed to filtered exhaust without particulate matter; and a group exposed to clean air. The exposure period was from day 7 until day 20 of pregnancy. In addition, 15 pregnant rats were treated with aromatase inhibitors or testosterone to clarify the process by which diesel exhaust exerts its toxicity. The anogenital distance was significantly longer in male and female fetuses from both exhaust-exposed groups than in those of the control. Differentiation of the testis, ovary, and thymus was delayed and disturbed. Maternal testosterone and progesterone levels, which increased due to pregnancy whether or not the rats were exposed, were significantly higher and lower, respectively, in the pregnant rats exposed to total exhaust and filtered exhaust. The serum adrenocorticotropic hormone (ACTH) level and urinary excretion of 17-hydroxycorticosteroids (OHCS) did not differ among the pregnant groups. These results indicate that elevated testosterone did not result from elevated maternal adrenal function. The fetoplacental-ovarian unit and inhibition of aromatase activity and synthesis caused by diesel exhaust inhalation might have played an essential role in the accumulation of testosterone. Since both exhaust-exposed groups showed almost the same reactions toward the inhalation, the gaseous phase must have included the relevant toxicants.

Neonatal development altered by maternal sulfur dioxide exposure.

Singh J.

Neurotoxicology. 1989 10(3):523-7.

Sulfur dioxide (SO₂) is one of the commonly encountered environmental contaminants. Experiments were carried out to test for neonatal behavioral alterations associated with maternal SO₂ exposure. Pregnant CD-1 mice were exposed to 0, 32, or 65 PPM SO₂ in environmental chambers from gestation day 7 to 18 (plug = day 1). The SO₂ air flow was set at 500 ml/min. Food and water were available at all times. The dams were allowed to deliver and neonatal behavioral development of the pups was studied. Maternal SO₂ exposure did not affect the mean number of live pups born/litter; however, the exposure at the high level significantly decreased the mean pup weight on day 1 of birth. SO₂ exposure at both levels significantly increased the time required for the righting reflex on day 1 of birth and negative geotaxis on day 10 of birth. The SO₂ exposure did not affect the aerial righting score of the pups on day 12 of birth. The data suggest that maternal SO₂ exposure can affect the neuromuscular coordination and may produce deficits in the functional capability of the developing offspring. The functional deficits appear to be associated with the birth weight of the offspring.

Effect of diesel exhaust emissions, particulates, and extract on sister chromatid exchange in transplacentally exposed fetal hamster liver.

Pereira M. A., McMillan L., Kaur P., Gulati D. K. and Sabharwal P. S.

Environmental Mutagenesis. 1982 4(3):215-20.

The genotoxic activity of diesel exhaust emissions, particulate matter, and an organic extract of the particulate matter was evaluated in transplacentally exposed Syrian hamster fetal liver cells. The frequency of sister chromatid exchange (SCE) was determined on day 13 of gestation. The extract of diesel particulate matter caused a dose-dependent increase in the frequency of SCE with a doubling in the incidence above 320mg/kg. The diesel particulate matter and diesel exhaust emissions did not alter the frequency of SCE. The extract and particulate matter did cause a dose-dependent decrease in the mitotic activity of the fetal liver. The in utero SCE analysis was demonstrated to be a sensitive assay for determination of the genotoxic activity of a complex mixture in transplacentally exposed fetuses.

Embryotoxicity of inhaled sulfur dioxide and carbon monoxide in mice and rabbits.

Murray F. J., Schwetz B. A., Crawford A. A., Henck J. W., Quast J. F. and Staples R. E.

Journal of Environmental Science and Health. Part C: Environmental Health Sciences. 1979 13(3):233-50.

The embryotoxic and teratogenic potential of sulfur dioxide (SO₂) was evaluated in CF-1 and New Zealand rabbits exposed to SO₂ alone or in combination with carbon monoxide (CO). The animals inhaled filtered room air (controls), SO₂ (mice, 25 ppm; rabbits, 70 ppm), or SO₂ plus CO (250 ppm) for 7 hr/day from days 6 through 15 (mice) and from days 6 through 18 (rabbits) of gestation. In both species, inhalation of SO₂ resulted in slight toxicity in the dams and an increased incidence of minor skeletal variants among their offspring; exposure to the

combination did not potentiate the increased incidence of these variants. A teratogenic effect was not discerned in either mice or rabbits exposed to SO₂ alone or in combination with carbon monoxide, but the fetuses of mice exposed to the combination were significantly smaller than those exposed only to SO₂.

B. Studies reporting no developmental or reproductive toxicity

Behavioural disturbances in adult CD-1 mice and absence of effects on their offspring upon SO₂ exposure.

Petruzzi S; Dell'Omo G; Fiore M; Chiarotti F; Bignami G, and Alleva E.
Archives of Toxicology. 1996 70(11):757-66.

Adult male and female CD-1 mice were exposed to different SO₂ concentrations (0,5,12, or 30 ppm) for 24 days, from 9 days before the formation of breeding pairs to pregnancy day 12-14. This exposure was near-continuous, covering about 80% of the total time indicated. The offspring of exposed dams were cross-fostered shortly after birth to dams not previously exposed. Videorecordings of the adult subjects' activities during the first hour after the start of exposure showed marked, acute transient behavioural effects such as increase of rearing and social interactions, which were more pronounced in males than in females. Subsequent activity tests on exposure days 3, 6, and 9 showed subacute effects including a dose-dependent decrease of grooming and an increase of digging as well as changes in chamber crossing and wall-rearing which were not dose-dependent; most of these effects were more pronounced in females than in males. Food and water consumption and body weight declined in a dose-dependent fashion only after the formation of breeding pairs, when consummatory responses were enhanced in the controls. Reproductive performance as well as postnatal somatic and neurobehavioural development of the offspring (the latter assessed by an observational test battery including eight reflexes and responses) were not affected by SO₂. Passive avoidance acquisition and retention at the young adult stage (60 days) and response changes produced by repeated apparatus exposure in non-reinforced animals (habituation) were similarly unaffected. Overall, the data indicate that SO₂ produces transient, acute behavioural disturbances and more subtle subacute response changes in adult mice which may be due, at least partly, to a functional interference with olfactory modulation of mouse behaviour. The absence of effects on reproductive performance and neurobehavioural development of the offspring suggests that the risk to the developing organism from gestational SO₂ exposure is low.

Teratological evaluation of sulfur dioxide.

Singh J.

Inst Environ Sci Proc. 1982 28:144-145.

Sulphur dioxide is a common air pollutant gas. Experiments were carried out to evaluate the teratogenicity of sulphur dioxide in mice. Pregnant animals were exposed to 0, 32, 65, 125 and 250 ppm of sulphur dioxide gas from gestation day 7-17. The animals were housed in plexiglass environmental chambers. The environmental chambers were equipped with an airtight see through door and to gascock openings at diagonal ends for quick flushing with gases. The concentrations of the gas was frequently monitored by Beckman Infrared Analyser Model 865. The gas mixture cylinders were obtained from union carbide and the cylinder were equipped with two stage regulators and micrometers. The animals were sacrificed on gestation day 18 and fetuses were studied for any teratological effects. A careful examination of the fetuses revealed that exposure to sulphur dioxide gas did not produce any significant effect on the number of dead or resorbed fetuses and the live pups did not show any significant teratological changes. However, a significant number of the fetuses had general hematomas at all level of exposure and spinal hematomas at low levels of exposure. [Notes: this is a two page report, grammar as in original.]

C. Related articles

In utero exposure to 1R4F reference cigarette smoke: evaluation of developmental toxicity.

Carmines E. L., Gaworski C. L., Faqi A. S. and Rajendran N.

Toxicological Sciences. 2003 75(1):134-47.

The potential developmental effects of 1R4F reference cigarette smoke were examined using Sprague-Dawley rats exposed for 2 h/day, 7 days/week, by nose-only inhalation at target mainstream smoke concentrations of 150, 300, and 600 mg/m³ total particulate matter (TPM). Males were exposed 4 weeks prior to and during mating, with females exposed 2 weeks prior to mating and during mating, and through gestation day (GD) 20. Sham controls received filtered air to simulate nose-only exposure, while cage controls were maintained untreated. Smoke exposure was confirmed through biomarker evaluation (parental: carboxyhemoglobin, nicotine, and cotinine; fetal: nicotine and cotinine). Characteristic cigarette smoke-related histopathologic changes including nasal epithelial hyperplasia and squamous metaplasia and pigmented macrophages in the lung were observed in all exposed parental groups. Maternal toxicity during gestation was indicated at smoke concentrations of 300 and 600 mg TPM/m³, where corrected total body weight gain was significantly ($p \leq 0.05$) decreased compared to sham controls. Food consumption was unaffected. Mating performance was unaffected by exposure: 71-75% pregnant in sham or cage control groups compared to 66-76% pregnant in smoke-exposed groups. Nicotine and cotinine were identified in the blood of fetuses from smoke-exposed dams on GD 20. Resorption rates, litter size, and sex ratio were equivalent among the groups. Fetal body weights at GD 20 were significantly reduced in the group exposed to 300 or 600 mg TPM/m³ compared to sham exposed controls. Developmental abnormalities were rare and

sporadic in nature and the absence of a clear smoke concentration relationship suggested they were not related to cigarette smoke inhalation. Skeletal examinations revealed delayed ossification (supra occipital and sternbrae) in fetuses from dams exposed to 300 or 600 mg TPM/m³ smoke. High concentrations of 1R4F cigarette smoke were not teratogenic. The methodology used for this study was able to detect a decrease in fetal birthweight and this approach may be a useful tool for cigarette evaluation.

In utero and lactational exposure to 1R4F cigarette smoke: effects on neonatal development, growth and neurobehavior in the offspring rats.

Gaworski C. L., Faqi A. S., Rajendran N. and Carmines E. L.
Toxicologist. 2003 72(S-1):75.

Potential pre- and postnatal effects of 1R4F cigarette smoke were examined using male and female Sprague-Dawley rats exposed 2 hrs/day 7 days/wk by nose-only inhalation at total particulate matter (TPM) concentrations of 150, 300, or 600 mg/m³ (males: 4-wks prior to and during mating, females: 2-wks prior to mating, during mating, and through lactation day 20). Sham controls received filtered air to simulate exposure conditions, while shelf controls were untreated. During lactation and until weaning (postnatal day: PND 61), the offspring rats were weighed, examined for gross signs of toxicity and evaluated for neurobehavioral effects. Biomarker analysis indicated smoke concentration related increases in blood COHb, nicotine and cotinine in exposed parental animals. Nicotine and cotinine were found in the pup blood during the lactation period. Characteristic cigarette smoke-related rodent respiratory tract histopathological changes were noted in all exposed parental groups. During gestation, maternal toxicity was indicated at smoke concentrations of 300 and 600 mg TPM/m³, where total maternal body weight gain was significantly (p less than or equal to 0.05) decreased compared to sham controls. Smoke-related effects in the offspring rats were noted at 600 mg TPM/m³, where pup body weights were significantly decreased at birth (approximately 17%), at weaning (approximately 18%) and at PND 61 (-12%). At 300 mg TPM/m³, significantly reduced pup weights were noted at birth but were consistent with sham controls thereafter. No adverse effects on developmental landmarks, age at vaginal patency or preputial separation, motor activity, acoustic startle response or learning and memory were observed in the offspring rats. This study indicates that maternal exposure to toxic levels of cigarette smoke during gestation, reduces birth weight, and retards growth in the rat. Cigarette smoke does not appear to produce developmental or neurobehavioral effects under the conditions of this test.

Placental transfer of metals of coal fly ash into various fetal organs of rat.

Srivastava V. K., Chauhan S. S., Srivastava P. K., Shukla R. R., Kumar V. and Misra U. K.
Archives of Toxicology. 1990 64(2):153-6.

Fly ash (100 mg/kg body weight) was administered intratracheally to 14-day pregnant rats for 6 consecutive days. On day 20 of gestation the translocation of metals present in the fly ash to various maternal and fetal organs was studied. Fly ash administration to pregnant mothers retarded the growth of fetal heart and kidney as determined by their weights. Fly ash instillation increased organ levels of nearly all the metals studied in both mother and fetus. Most of the

metals present in coal fly ash were transferred in significant amounts through placenta to several fetal organs. However, the pattern of their distribution into various fetal organs was different for different metals.

TRIHALOMETHANES

This is a compilation of abstracts of articles identified during the preliminary toxicological evaluation of evidence on the developmental and reproductive toxicology of the chemical group Trihalomethanes (THMs). THMs are produced mainly as byproducts of water disinfection with halogenated compounds. The most common THMs are: bromodichloromethane (BDCM, CAS# 75-27-4), dibromochloromethane (DBCM, CAS# 124-48-1), tribromomethane (TBM, bromoform, CAS #75-25-2) and trichloromethane (TCM, chloroform, CAS# 67-66-3). Trihalomethanes that can form with other halogens such as iodine and fluorine are not included in this compilation.

Compiled are abstracts from developmental and reproductive epidemiologic and animal toxicity studies and other relevant investigations. The epidemiologic studies report on developmental and reproductive sequelae related to exposure to disinfection by-products in drinking water.

- There were a total of fifty-eight identified studies in the search for THMs and each of the four most common members of the group. These are summarized in Table 1.
- Six epidemiologic studies showed increased risk of adverse developmental or reproductive outcomes for THMs. Five studies reported no increased risk. Eleven other related studies or meeting presentations were identified. With regard to specific THMs, epidemiologic studies found are as follows:
 - BDCM: Four studies showing increased risk and four studies reporting no increased risk of adverse developmental or reproductive outcomes were identified. Four related articles or meeting abstracts on BDCM were identified.
 - DBCM: One study showing increased risk and one study reporting no increased risk of adverse developmental or reproductive outcomes and one related study were identified.
 - TBM: Two studies reported no increased risk of adverse developmental or reproductive outcomes.
 - TCM: Five studies reporting increased risk and three studies reporting no increased risk of adverse developmental or reproductive outcomes as well as four related or meeting abstracts were identified.
- Two animal studies of THMs reported increased risk and one study reported no increased risk of adverse developmental or reproductive outcomes. Six animal related and/or meeting abstracts were also identified for THMs. With regard to specific THMs tested in animals:
 - BDCM: Five animal studies reported increased risk and five reported no increased risk of adverse developmental or reproductive outcomes. Nine related articles or meeting abstracts were identified.
 - DBCM: There were no animal studies identified.

- TBM: Two studies reported increased risk and three reported no increased risk of adverse developmental or reproductive outcomes. There were three related articles or meeting abstracts.
- TCM: Three studies reported increased risk of adverse developmental or reproductive outcomes and one related article or meeting abstract was identified.

Table 1: Summary of the identified studies on the developmental and reproductive toxicity of THMs

Category	THM	BDCM	DBCm	TBM	TCM	Total number of studies*
Human studies						
Reporting increased risk	6	4	1	0	5	9
Reporting no increased risk	5	4	1	2	3	9***
Related studies and meeting abstracts	11	4	1	0	4**	15
Animal studies						
Positive	2	5	0	2	3	8
Negative	1	5	0	3	0	6***
Related studies and meeting abstracts	6	9	0	3	1	13
TOTAL						60***

* Number of studies in each category

** Includes one study without an abstract

*** Includes one study that is in two different categories; therefore the total number of studies is 58 (=60-2).

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I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

A.1 Total Trihalomethanes

Water disinfection by-products and the risk of specific birth defects: a population-based cross-sectional study in Taiwan.

Hwang B. F., Jaakkola J. J. and Guo H. R.
Environmental Health. 2008;7:23.

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. METHODS: 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 eleven most common specific defects in four disinfection by-product exposure categories based on the levels of total trihalomethanes (TTHMs) representing high (TTHMs 20+ microg/L), medium (TTHMs 10-19 microg/L), low exposure (TTHMs 5-9 microg/L), and 0-4 microg/L as the reference category. In addition, we conducted a meta-analysis of the results from the 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 palate (1.56, 95% CI: 1.00, 2.41), and anencephalus (1.96, 95% CI: 0.94, 4.07) were elevated in the high exposure compared to the reference category. In the meta-analysis, the summary odds ratio for ventricular septal defects (1.59, 95% CI: 1.21, 2.07) was consistently elevated. CONCLUSION: The present study suggests that prenatal exposure to disinfection by-products increases the risk of ventricular septal defects, cleft palate, and anencephalus. The evidence on ventricular septal defects is consistent in the three available studies.

Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England.

Toledano M. B., Nieuwenhuijsen M. J., Best N., Whitaker H., Hambly P., de H. C., Fawell J., Jarup L. and Elliott P.
Environmental Health Perspectives. 2005 113(2):225-32.

We investigated the association between total trihalomethanes (TTHMs) and risk of stillbirth and low and very low birth weight in three water regions in England, 1992-1998; associations with individual trihalomethanes (THMs) were also examined. Modeled estimates of quarterly TTHM concentrations in water zones, categorized as low (< 30 microg/L), medium (30-59 microg/L), or high (> or = 60 microg/L), were linked to approximately 1 million routine birth and stillbirth

records using maternal residence at time of birth. In one region, where there was a positive socioeconomic deprivation gradient across exposure categories, there was also a positive, significant association of TTHM with risk of stillbirth and low and very low birth weight. Overall summary estimates across the three regions using a random-effects model to allow for between-region heterogeneity in exposure effects showed small excess risks in areas with high TTHM concentrations for stillbirths [odds ratio (OR) = 1.11; 95% confidence interval (CI), 1.00-1.23), low birth weight (OR = 1.09; 95% CI, 0.93-1.27), and very low birth weight (OR = 1.05; 95% CI, 0.82-1.34). Among the individual THMs, chloroform showed a similar pattern of risk as TTHM, but no association was found with concentrations of bromodichloromethane or total brominated THMs. Our findings overall suggest a significant association of stillbirths with maternal residence in areas with high TTHM exposure. Further work is needed looking at cause-specific stillbirths and effects of other disinfection by-products and to help differentiate between alternative (noncausal) explanations and those that may derive from the water supply.

The effect of disinfection by-products and mutagenic activity on birth weight and gestational duration.

Wright J. M., Schwartz J. and Dockery D. W.

Environmental Health Perspectives. 2004 112(8):920-5.

Epidemiologic studies of disinfection by-products have traditionally focused on total trihalomethane (TTHM) concentration as a surrogate for maternal exposure during pregnancy. We used birth certificate data on 196,000 infants to examine the effect of third-trimester exposures on various indices of fetal development. We examined the effect of town-average concentrations of TTHM and additional exposure metrics in relation to mean birth weight, mean gestational age, small for gestational age (SGA) infancy, and preterm delivery. Trihalomethane data (TTHM, chloroform, and bromodichloromethane) from 1995-1998 were available for 109 towns in Massachusetts. Data from 1997-1998 on haloacetic acid (total haloacetic acids, dichloroacetic acid, and trichloroacetic acid), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), and mutagenicity were available for a limited number of towns. We observed reductions in mean birth weight (12-18 g) for maternal trihalomethane exposures > the 90th percentile compared with those < the 50th percentile. Birth weight reductions were detected for chloroform exposures > 20 microg/L and TTHM exposures > 40 microg/L. Elevated trihalomethanes were associated with increases in gestational duration and a reduced risk of preterm delivery. We found evidence of an exposure-response effect of trihalomethanes on risk of SGA, with odds ratios (ORs) ranging from 1.09 to 1.23 for bromodichloromethane exposures > 5 microg/L. Elevated mutagenic activity was associated with SGA [OR = 1.25; 95% confidence interval (CI), 1.04 to 1.51] and mean birth weight (-27 g; 95% CI, -54 to -1). Although smaller in magnitude, our findings are consistent with previous studies reporting associations between trihalomethanes and SGA. These data also suggest a relationship between fetal development indices and mutagenic activity independent of exposure to trihalomethanes, haloacetic acids, and MX.

Chlorination by-products in drinking water and menstrual cycle function.

Windham G. C., Waller K., Anderson M., Fenster L., Mendola P. and Swan S.

Environmental Health Perspectives. 2003 111(7):935-41; discussion A409.

We analyzed data from a prospective study of menstrual cycle function and early pregnancy loss to explore further the effects of trihalomethanes (THM) on reproductive end points. Premenopausal women (*n* = 403) collected urine samples daily during an average of 5.6 cycles for measurement of steroid metabolites that were used to define menstrual parameters such as cycle and phase length. Women were asked about consumption of various types of water as well as other habits and demographics. A THM level was estimated for each cycle based on residence and quarterly measurements made by water utilities during a 90-day period beginning 60 days before the cycle start date. We found a monotonic decrease in mean cycle length with increasing total THM (TTHM) level; at > 60 microg/L, the adjusted decrement was 1.1 days [95% confidence interval (CI), -1.8 to -0.40], compared with less than or equal to 40 microg/L. This finding was also reflected as a reduced follicular phase length (difference - 0.94 day; 95% CI, -1.6 to -0.24). A decrement in cycle and follicular phase length of 0.18 days (95% CI, -0.29 to -0.07) per 10 microg/L unit increase in TTHM concentration was found. There was little association with luteal phase length, menses length, or cycle variability. Examining the individual THMs by quartile, we found the greatest association with chlorodibromomethane or the sum of the brominated compounds. Incorporating tap water consumption showed a similar pattern of reduced cycle length with increasing TTHM exposure. These findings suggest that THM exposure may affect ovarian function and should be confirmed in other studies.

Relation between stillbirth and specific chlorination by-products in public water supplies.

King W. D., Dodds L. and Allen A. C.

Environmental Health Perspectives. 2000 108(9):883-6.

During water treatment, chlorine reacts with naturally occurring organic matter in surface water to produce a number of by-products. Of the by-products formed, trihalomethanes (THMs) are among the highest in concentration. We conducted a retrospective cohort study to evaluate the relationship between the level of total THM and specific THMs in public water supplies and risk for stillbirth. The cohort was assembled from a population-based perinatal database in the Canadian province of Nova Scotia and consisted of almost 50,000 singleton deliveries between 1988 and 1995. Individual exposures were assigned by linking mother's residence at the time of delivery to the levels of specific THMs monitored in public water supplies. Analysis was conducted for all stillbirths and for cause-of-death categories based on the physiologic process responsible for the fetal death. Total THMs and the specific THMs were each associated with increased stillbirth risk. The strongest association was observed for bromodichloromethane exposure, where risk doubled for those exposed to a level of [greater and equal to] 20 microg/L compared to those exposed to a level < 5 microg/L (relative risk = 1.98, 95% confidence interval, 1.23-3.49). Relative risk estimates associated with THM exposures were larger for asphyxia-related deaths than for unexplained deaths or for stillbirths overall. These findings suggest a need to consider specific chlorination by-products in relation to stillbirth risk, in particular bromodichloromethane and other by-product correlates. The finding of a stronger effect for asphyxia deaths requires confirmation and research into possible mechanisms.

Trihalomethanes in drinking water and spontaneous abortion.

Waller K., Swan S. H., DeLorenze G. and Hopkins B.
Epidemiology. 1998 9(2):134-40.

Trihalomethanes (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) are common contaminants of chlorinated drinking water. Although animal data indicate that these compounds may be reproductive toxicants, little information exists on their relation to spontaneous abortion in humans. We examined exposure to trihalomethanes and spontaneous abortion in a prospective study of 5,144 pregnant women in a prepaid health plan. Seventy-eight drinking water utilities provided concurrent trihalomethane sampling data. We calculated total trihalomethane levels by averaging all measurements taken by the subject's utility during her first trimester. We calculated exposures to individual trihalomethanes in an analogous manner. Women who drank ≥ 5 glasses per day of cold tapwater containing ≥ 75 micrograms per liter total trihalomethanes had an adjusted odds ratio (OR) of 1.8 for spontaneous abortion [95% confidence interval (CI) = 1.1-3.0]. Of the four individual trihalomethanes, only high bromodichloromethane exposure (consumption of ≥ 5 glasses per day of cold tapwater containing ≥ 18 micrograms per liter bromodichloromethane) was associated with spontaneous abortion both alone (adjusted OR = 2.0; 95% CI = 1.2-3.5) and after adjustment for the other trihalomethanes (adjusted OR = 3.0; 95% CI = 1.4-6.6).

A.2 BDCM

The effect of disinfection by-products and mutagenic activity on birth weight and gestational duration.

Wright J. M., Schwartz J. and Dockery D. W.
Environmental Health Perspectives. 2004 112(8):920-5.

Epidemiologic studies of disinfection by-products have traditionally focused on total trihalomethane (TTHM) concentration as a surrogate for maternal exposure during pregnancy. We used birth certificate data on 196,000 infants to examine the effect of third-trimester exposures on various indices of fetal development. We examined the effect of town-average concentrations of TTHM and additional exposure metrics in relation to mean birth weight, mean gestational age, small for gestational age (SGA) infancy, and preterm delivery. Trihalomethane data (TTHM, chloroform, and bromodichloromethane) from 1995-1998 were available for 109 towns in Massachusetts. Data from 1997-1998 on haloacetic acid (total haloacetic acids, dichloroacetic acid, and trichloroacetic acid), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), and mutagenicity were available for a limited number of towns. We observed reductions in mean birth weight (12-18 g) for maternal trihalomethane exposures $>$ the 90th percentile compared with those $<$ the 50th percentile. Birth weight reductions were detected for chloroform exposures > 20 microg/L and TTHM exposures > 40 microg/L. Elevated trihalomethanes were associated with increases in gestational duration and a reduced risk of preterm delivery. We found evidence of an exposure-response effect of trihalomethanes on risk of SGA, with odds ratios (ORs) ranging from 1.09 to 1.23 for bromodichloromethane exposures > 5 microg/L. Elevated mutagenic activity was associated with SGA [OR = 1.25; 95%

confidence interval (CI), 1.04 to 1.51] and mean birth weight (-27 g; 95% CI, -54 to -1). Although smaller in magnitude, our findings are consistent with previous studies reporting associations between trihalomethanes and SGA. These data also suggest a relationship between fetal development indices and mutagenic activity independent of exposure to trihalomethanes, haloacetic acids, and MX.

Relation between trihalomethane compounds and birth defects.

Dodds L. and King W. D.

Occupational and Environmental Medicine. 2001 58(7):443-6.

OBJECTIVES: To evaluate the risk of birth defects relative to exposure to specific trihalomethanes in public water supplies. **METHODS:** A retrospective cohort study was conducted based on data from a population based perinatal database in Nova Scotia, Canada and from the results of routine water monitoring tests. The cohort consisted of women who had a singleton birth in Nova Scotia between 1988 and 1995 and who lived in an area with a municipal water supply. The birth defects analyzed included neural tube defects, cardiovascular defects, cleft defects, and chromosomal abnormalities. Two of the four trihalomethane compounds occur in large enough concentrations to be analyzed (chloroform and bromodichloromethane (BDCM)). **RESULTS:** Exposure to BDCM at concentrations of 20 microg/l or over was associated with an increased risk of neural tube defects (adjusted relative risk (RR) 2.5, 95% confidence interval (95% CI) 1.2 to 5.1) whereas exposure to chloroform was not. Exposure to BDCM of 20 microg/l and over was associated with decreased risks of cardiovascular anomalies (RR 0.3, 95% CI 0.2 to 0.7). There was a suggestion of an increased risk of chromosomal abnormalities associated with exposure to chloroform, and no evidence of any association between either trihalomethane compound and cleft defects. **CONCLUSIONS:** In this cohort, differences were found in the RR associated with exposure to chloroform and BDCM for each of the congenital anomalies under study. These findings point to the importance of examining specific byproduct compounds relative to risk for these birth outcomes and in particular implicate BDCM and other correlated disinfection byproducts in the aetiology of neural tube defects.

Relation between stillbirth and specific chlorination by-products in public water supplies.

King W. D., Dodds L. and Allen A. C.

Environmental Health Perspectives. 2000 108(9):883-6.

During water treatment, chlorine reacts with naturally occurring organic matter in surface water to produce a number of by-products. Of the by-products formed, trihalomethanes (THMs) are among the highest in concentration. We conducted a retrospective cohort study to evaluate the relationship between the level of total THM and specific THMs in public water supplies and risk for stillbirth. The cohort was assembled from a population-based perinatal database in the Canadian province of Nova Scotia and consisted of almost 50,000 singleton deliveries between 1988 and 1995. Individual exposures were assigned by linking mother's residence at the time of delivery to the levels of specific THMs monitored in public water supplies. Analysis was conducted for all stillbirths and for cause-of-death categories based on the physiologic process

responsible for the fetal death. Total THMs and the specific THMs were each associated with increased stillbirth risk. The strongest association was observed for bromodichloromethane exposure, where risk doubled for those exposed to a level of [greater and equal to] 20 microg/L compared to those exposed to a level < 5 microg/L (relative risk = 1.98, 95% confidence interval, 1.23-3.49). Relative risk estimates associated with THM exposures were larger for asphyxia-related deaths than for unexplained deaths or for stillbirths overall. These findings suggest a need to consider specific chlorination by-products in relation to stillbirth risk, in particular bromodichloromethane and other by-product correlates. The finding of a stronger effect for asphyxia deaths requires confirmation and research into possible mechanisms.

Trihalomethanes in drinking water and spontaneous abortion.

Waller K., Swan S. H., DeLorenze G. and Hopkins B.
Epidemiology 1998 9(2):134-40.

Trihalomethanes (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) are common contaminants of chlorinated drinking water. Although animal data indicate that these compounds may be reproductive toxicants, little information exists on their relation to spontaneous abortion in humans. We examined exposure to trihalomethanes and spontaneous abortion in a prospective study of 5,144 pregnant women in a prepaid health plan. Seventy-eight drinking water utilities provided concurrent trihalomethane sampling data. We calculated total trihalomethane levels by averaging all measurements taken by the subject's utility during her first trimester. We calculated exposures to individual trihalomethanes in an analogous manner. Women who drank > or = 5 glasses per day of cold tapwater containing > or = 75 micrograms per liter total trihalomethanes had an adjusted odds ratio (OR) of 1.8 for spontaneous abortion [95% confidence interval (CI) = 1.1-3.0]. Of the four individual trihalomethanes, only high bromodichloromethane exposure (consumption of > or = 5 glasses per day of cold tapwater containing > or = 18 micrograms per liter bromodichloromethane) was associated with spontaneous abortion both alone (adjusted OR = 2.0; 95% CI = 1.2-3.5) and after adjustment for the other trihalomethanes (adjusted OR = 3.0; 95% CI = 1.4-6.6).

A.3 DBCM

Chlorination by-products in drinking water and menstrual cycle function.

Windham G. C., Waller K., Anderson M., Fenster L., Mendola P. and Swan S.
Environmental Health Perspectives. 2003 111(7):935-41; discussion A409.

We analyzed data from a prospective study of menstrual cycle function and early pregnancy loss to explore further the effects of trihalomethanes (THM) on reproductive end points. Premenopausal women (*n* = 403) collected urine samples daily during an average of 5.6 cycles for measurement of steroid metabolites that were used to define menstrual parameters such as cycle and phase length. Women were asked about consumption of various types of water as well as other habits and demographics. A THM level was estimated for each cycle based on residence and quarterly measurements made by water utilities during a 90-day period beginning 60 days before the cycle start date. We found a monotonic decrease in mean

cycle length with increasing total THM (TTHM) level; at > 60 microg/L, the adjusted decrement was 1.1 days [95% confidence interval (CI), -1.8 to -0.40], compared with less than or equal to 40 microg/L. This finding was also reflected as a reduced follicular phase length (difference - 0.94 day; 95% CI, -1.6 to -0.24). A decrement in cycle and follicular phase length of 0.18 days (95% CI, -0.29 to -0.07) per 10 microg/L unit increase in TTHM concentration was found. There was little association with luteal phase length, menses length, or cycle variability. Examining the individual THMs by quartile, we found the greatest association with chlorodibromomethane or the sum of the brominated compounds. Incorporating tap water consumption showed a similar pattern of reduced cycle length with increasing TTHM exposure. These findings suggest that THM exposure may affect ovarian function and should be confirmed in other studies.

A.4 Bromoform

No abstracts were identified in this category.

A.5 Chloroform

Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England.

Toledano M. B., Nieuwenhuijsen M. J., Best N., Whitaker H., Hambly P., de H. C., Fawell J., Jarup L. and Elliott P.

Environmental Health Perspectives. 2005 113(2):225-32.

We investigated the association between total trihalomethanes (TTHMs) and risk of stillbirth and low and very low birth weight in three water regions in England, 1992-1998; associations with individual trihalomethanes (THMs) were also examined. Modeled estimates of quarterly TTHM concentrations in water zones, categorized as low (< 30 microg/L), medium (30-59 microg/L), or high (> or = 60 microg/L), were linked to approximately 1 million routine birth and stillbirth records using maternal residence at time of birth. In one region, where there was a positive socioeconomic deprivation gradient across exposure categories, there was also a positive, significant association of TTHM with risk of stillbirth and low and very low birth weight. Overall summary estimates across the three regions using a random-effects model to allow for between-region heterogeneity in exposure effects showed small excess risks in areas with high TTHM concentrations for stillbirths [odds ratio (OR) = 1.11; 95% confidence interval (CI), 1.00-1.23], low birth weight (OR = 1.09; 95% CI, 0.93-1.27), and very low birth weight (OR = 1.05; 95% CI, 0.82-1.34). Among the individual THMs, chloroform showed a similar pattern of risk as TTHM, but no association was found with concentrations of bromodichloromethane or total brominated THMs. Our findings overall suggest a significant association of stillbirths with maternal residence in areas with high TTHM exposure. Further work is needed looking at cause-specific stillbirths and effects of other disinfection by-products and to help differentiate between alternative (noncausal) explanations and those that may derive from the water supply.

The effect of disinfection by-products and mutagenic activity on birth weight and gestational duration.

Wright J. M., Schwartz J. and Dockery D. W.

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Epidemiologic studies of disinfection by-products have traditionally focused on total trihalomethane (TTHM) concentration as a surrogate for maternal exposure during pregnancy. We used birth certificate data on 196,000 infants to examine the effect of third-trimester exposures on various indices of fetal development. We examined the effect of town-average concentrations of TTHM and additional exposure metrics in relation to mean birth weight, mean gestational age, small for gestational age (SGA) infancy, and preterm delivery. Trihalomethane data (TTHM, chloroform, and bromodichloromethane) from 1995-1998 were available for 109 towns in Massachusetts. Data from 1997-1998 on haloacetic acid (total haloacetic acids, dichloroacetic acid, and trichloroacetic acid), 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), and mutagenicity were available for a limited number of towns. We observed reductions in mean birth weight (12-18 g) for maternal trihalomethane exposures > the 90th percentile compared with those < the 50th percentile. Birth weight reductions were detected for chloroform exposures > 20 microg/L and TTHM exposures > 40 microg/L. Elevated trihalomethanes were associated with increases in gestational duration and a reduced risk of preterm delivery. We found evidence of an exposure-response effect of trihalomethanes on risk of SGA, with odds ratios (ORs) ranging from 1.09 to 1.23 for bromodichloromethane exposures > 5 microg/L. Elevated mutagenic activity was associated with SGA [OR = 1.25; 95% confidence interval (CI), 1.04 to 1.51] and mean birth weight (-27 g; 95% CI, -54 to -1). Although smaller in magnitude, our findings are consistent with previous studies reporting associations between trihalomethanes and SGA. These data also suggest a relationship between fetal development indices and mutagenic activity independent of exposure to trihalomethanes, haloacetic acids, and MX.

Pregnancy outcome of personnel in Swedish biomedical research laboratories.

Wennborg H., Bodin L., Vainio H. and Axelsson G.

Journal of Occupational and Environmental Medicine. 2000 42(4):438-46.

Possible hazardous effects of laboratory work on the reproduction outcomes of female laboratory personnel in Sweden from 1990 to 1994 were investigated in a questionnaire-based study (n = 1052) by comparison with personnel in non-laboratory departments. The individual woman constituted the primary sampling unit, with her pregnancies defined as the unit of analysis. Allowance for dependence between different pregnancies of the same woman was considered by applying random effect models. With regard to spontaneous abortions, no elevated odds ratio was found for laboratory work in general, but an odds ratio of 2.3 and a 95% confidence interval of 0.9 to 5.9 (n = 856) was connected to working with chloroform. The odds ratio for large for gestational age infants in association with the mother's laboratory work was 1.9 (confidence interval, 0.7 to 5.2). The result with regard to spontaneous abortion partly supports previously reported increased risks of miscarriage related to laboratory work with solvents.

The association of waterborne chloroform with intrauterine growth retardation.

Kramer M. D., Lynch C. F., Isacson P. and Hanson J. W.
Epidemiology 1992 3(5):407-13.

The potential reproductive effects of long-term, low-dose exposure to chloroform have received little attention despite the known, acute toxicity of high exposures and the wide-spread occurrence of low concentrations in drinking water. We studied the association of waterborne chloroform with low birthweight (less than 2,500 gm), prematurity (less than 37 weeks gestation), and intrauterine growth retardation (less than 5th percentile of weight for gestational age). Cases were not mutually exclusive, but each outcome was analyzed independently. Birth certificates from January 1, 1989, to June 30, 1990, were used to identify cases and randomly selected controls. All were live, singleton infants born to non-Hispanic, white women from Iowa towns with 1,000-5,000 inhabitants. Exposures to chloroform and other trihalomethanes were ecologic variables based on maternal residence and a 1987 municipal water survey. After adjustment for maternal age, parity, adequacy of prenatal care, marital status, education, and maternal smoking by multiple logistic regression, residence in municipalities where chloroform concentrations were greater than or equal to 10 micrograms/liter was associated with an increased risk for intrauterine growth retardation (odds ratio = 1.8, 95% confidence interval = 1.1-2.9). The major limitations of this study involve the ascertainment and classification of exposures to trihalomethanes, including such issues as the imprecision of using aggregate municipal measures for classifying exposure at the level of the individual, the potential misclassification due to residential mobility, and the fluctuation of trihalomethane levels.

Association between waterborne chloroform and intrauterine growth retardation.

Kramer M. D., Lynch C. F., Isacson P. and Hanson J. W.
American Journal of Epidemiology. 1991 134(7):722.

The health effects of long-term, low-dose exposure to chloroform have not been adequately investigated. Low birth weight (less than 2,500 g), prematurity (less than 37 weeks gestation), and intrauterine growth retardation (less than 5th percentile of weight for gestational age) were evaluated independently for evidence that low doses of chloroform in public water supplies might be a contributing factor. Each case infant met at least one of these definitions. Case infants and randomly selected control infants (1:5) were live, singleton infants (identified from birth certificates dated from January 1, 1989 to June 30, 1990) who were born to non-Hispanic, white women in Iowa town with 1,000 to 5,000 inhabitants. Chloroform exposure was an ecologic variable based on maternal residence and a 1987 municipal water survey. After adjusting by logistic regression for maternal age, parity, adequacy of prenatal care, marital status, education, and maternal smoking, chloroform (greater than or equal to 10 ug/liter) was associated with an increased risk for intrauterine growth retardation (odds ratio = 1.8, 95% confidence interval 1.1-2.9). Prematurity and low birth weight were not significantly associated with chloroform exposure. Public water supplies containing elevated chloroform levels may increase the risk of intrauterine growth retardation. Studies incorporating direct measures of chloroform exposure at the individual level should be conducted.

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

B.1 Total Trihalomethanes

Chlorination disinfection by-products and risk of congenital anomalies in England and Wales.

Nieuwenhuijsen M. J., Toledano M. B., Bennett J., Best N., Hambly P., de H. C., Wellesley D., Boyd P. A., Abramsky L., Dattani N., Fawell J., Briggs D., Jarup L. and Elliott P. Environmental Health Perspectives. 2008 116(2):216-22.

BACKGROUND: Increased risk of various congenital anomalies has been reported to be associated with trihalomethane (THM) exposure in the water supply. **OBJECTIVES:** We conducted a registry-based study to determine the relationship between THM concentrations and the risk of congenital anomalies in England and Wales. **METHODS:** We obtained congenital anomaly data from the National Congenital Anomalies System, regional registries, and the national terminations registry; THM data were obtained from water companies. Total THM (< 30, 30 to < 60, > or =60 microg/L), total brominated exposure (< 10, 10 to < 20, > or =20 microg/L), and bromoform exposure (< 2, 2 to < 4, > or =4 microg/L) were modeled at the place of residence for the first trimester of pregnancy. We included 2,605,226 live births, stillbirths, and terminations with 22,828 cases of congenital anomalies. Analyses using fixed- and random-effects models were performed for broadly defined groups of anomalies (cleft palate/lip, abdominal wall, major cardiac, neural tube, urinary and respiratory defects), a more restricted set of anomalies with better ascertainment, and for isolated and multiple anomalies. Data were adjusted for sex, maternal age, and socioeconomic status. **RESULTS:** We found no statistically significant trends across exposure categories for either the broadly defined or more restricted sets of anomalies. For the restricted set of anomalies with isolated defects, there were significant ($p < 0.05$) excess risks in the high-exposure categories of total THMs for ventricular septal defects [odds ratio (OR) = 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). **CONCLUSION:** In this large national study we found little evidence for a relationship between THM concentrations in drinking water and risk of congenital anomalies.

Exposure to drinking water disinfection by-products and pregnancy loss.

Savitz D. A., Singer P. C., Herring A. H., Hartmann K. E., Weinberg H. S. and Makarushka C. American Journal of Epidemiology. 2006 164(11):1043-51.

Previous research has suggested that exposure to elevated levels of drinking water disinfection by-products (DBPs) may cause pregnancy loss. In 2000-2004, the authors conducted a study in three US locations of varying DBP levels and evaluated 2,409 women in early pregnancy to assess their tap water DBP concentrations, water use, other risk factors, and pregnancy outcome. Tap water concentrations were measured in the distribution system weekly or biweekly. The authors considered DBP concentration and ingested amount and, for trihalomethanes only,

bathing/showering and integrated exposure that included ingestion. On the basis of 258 pregnancy losses, they did not find an increased risk of pregnancy loss in relation to trihalomethane, haloacetic acid, or total organic halide concentrations; ingested amounts; or total exposure. In contrast to a previous study, pregnancy loss was not associated with high personal trihalomethane exposure (≥ 75 micro g/liter and ≥ 5 glasses of water/day) (odds ratio = 1.1, 95% confidence interval: 0.7, 1.7). Sporadic elevations in risk were found across DBPs, most notably for ingested total organic halide (odds ratio = 1.5, 95% confidence interval: 1.0, 2.2 for the highest exposure quintile). These results provide some assurance that drinking water DBPs in the range commonly encountered in the United States do not affect fetal survival.

Late pregnancy exposures to disinfection by-products and growth-related birth outcomes.

Hinckley A. F., Bachand A. M. and Reif J. S.

Environmental Health Perspectives. 2005 113(12):1808-13.

Toxicologic studies have demonstrated associations between growth-related birth outcomes and exposure to high concentrations of disinfection by-products (DBPs), including specific trihalomethane (THM) and haloacetic acid (HAA) chemical subspecies. Few prior investigations of DBPs have evaluated exposure during the third trimester of pregnancy, the time period of gestation when fetal growth may be most sensitive to environmental influences. We conducted a retrospective cohort study to examine the effects of exposure to THMs and HAAs during the third trimester and during individual weeks and months of late gestation on the risks for term low birth weight, intrauterine growth retardation, and very preterm and preterm births. The study population (n = 48,119) included all live births and fetal deaths occurring from January 1998 through March 2003 to women whose residence was served by one of three community water treatment facilities. We found evidence of associations between exposure to specific HAAs and term low birth weight as well as intrauterine growth retardation and for exposure to the five regulated HAAs (HAA5) and term low birth weight. Our findings suggest a critical window of exposure with respect to fetal development during weeks 33-40 for the effects of dibromoacetic acid and during weeks 37-40 for the effects of dichloroacetic acid. Adjustment for potential confounders did not affect the conclusions.

The effect of trihalomethane and haloacetic acid exposure on fetal growth in a Maryland county.

Porter C. K., Putnam S. D., Hunting K. L. and Riddle M. R.

American Journal of Epidemiology. 2005 15;162(4):334-44.

As water flows from treatment plants to the tap, chlorine, used to disinfect surface water meant for residential use, reacts with residual organic and inorganic matter, creating chlorine disinfection by-products. In recent years, these by-products have been scrutinized as a potential reproductive and developmental hazard. This study examined whether exposure to the four total trihalomethanes or the five haloacetic acids (two major subgroups of chlorine disinfection by-products) was related to an increased risk of intrauterine growth retardation in four regions of a Maryland county from 1998 to 2002. Maternal exposure to each by-product was evaluated for each trimester as well as over the entire pregnancy. The authors were not able to demonstrate any

consistent, statistically significant effect on intrauterine growth retardation associated with any of the chlorine disinfection by-products, nor did they find any indication of a dose-response relation. However, they did find some potential for a slightly elevated risk of intrauterine growth retardation during the second and third trimesters for both total trihalomethanes and five haloacetic acids when comparing increasing quintiles of exposure to constituents of total trihalomethanes and five haloacetic acids.

Abruptio placentae and disinfection by-products in the public water supply of Ontario, Canada.

Broers T., King W. D., Arbuckle T. E. and Liu S.
American Journal of Epidemiology 2001 153(11).

Recent epidemiologic studies on disinfection by-products (DBPs) have suggested elevated risks for fetal death, but have not considered the placental conditions that are often a cause. This retrospective cohort study examines the association between DBPs and abruptio placenta, the most common cause of intrapartum fetal death. A national hospital discharge database was used to identify a cohort of women who had singleton live and stillbirth deliveries in Ontario between 1990 and 1997 and were living in a municipality serviced by a public water supply system (n = 695,857). Postal codes were used to identify the water treatment facility serving each subject. Monthly DBP concentrations for each treatment facility were interpolated from quarterly data and average DBP concentrations for the four-month period surrounding the estimated date of conception were used to calculate individual exposure to specific DBP compounds. Relationships were examined using relative risks (RR) with 95% confidence intervals (CIs). No associations were observed between abruptio placenta and total trihalomethanes or bromodichloromethane. Elevated risks were detected between total haloacetic acid levels of 40 ug/liter or greater versus a level of under 10 ug/liter among all abruptio placenta cases (RR = 1.21; 95% CI = 1.08-1.34) and those abruptio placentae cases that ended in stillbirth (RR = 2.02; 95% CI = 1.64-2.40). Further investigation into haloacetic acid is needed to corroborate these findings.

B.2 BDCM

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trihalomethane, haloacetic acid, or total organic halide concentrations; ingested amounts; or total exposure. In contrast to a previous study, pregnancy loss was not associated with high personal trihalomethane exposure (≥ 75 micro g/liter and ≥ 5 glasses of water/day) (odds ratio = 1.1, 95% confidence interval: 0.7, 1.7). Sporadic elevations in risk were found across DBPs, most notably for ingested total organic halide (odds ratio = 1.5, 95% confidence interval: 1.0, 2.2 for the highest exposure quintile). These results provide some assurance that drinking water DBPs in the range commonly encountered in the United States do not affect fetal survival.

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B.3 DBCM

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B.4 Bromoform

Chlorination disinfection by-products and risk of congenital anomalies in England and Wales.

Nieuwenhuijsen M. J., Toledano M. B., Bennett J., Best N., Hambly P., de H. C., Wellesley D., Boyd P. A., Abramsky L., Dattani N., Fawell J., Briggs D., Jarup L. and Elliott P. Environmental Health Perspectives 2008 116(2):216-22.

BACKGROUND: Increased risk of various congenital anomalies has been reported to be associated with trihalomethane (THM) exposure in the water supply. **OBJECTIVES:** We conducted a registry-based study to determine the relationship between THM concentrations and the risk of congenital anomalies in England and Wales. **METHODS:** We obtained congenital anomaly data from the National Congenital Anomalies System, regional registries, and the national terminations registry; THM data were obtained from water companies. Total THM (< 30 , 30 to < 60 , ≥ 60 microg/L), total brominated exposure (< 10 , 10 to < 20 , ≥ 20 microg/L), and bromoform exposure (< 2 , 2 to < 4 , ≥ 4 microg/L) were modeled at the place of residence for the first trimester of pregnancy. We included 2,605,226 live births, stillbirths, and terminations with 22,828 cases of congenital anomalies. Analyses using fixed- and random-effects models were performed for broadly defined groups of anomalies (cleft palate/lip, abdominal wall, major cardiac, neural tube, urinary and respiratory defects), a more restricted set of anomalies with better ascertainment, and for isolated and multiple anomalies. Data were adjusted for sex, maternal age, and socioeconomic status. **RESULTS:** We found no statistically significant trends across exposure categories for either the broadly defined or more restricted sets of anomalies. For the restricted set of anomalies with isolated defects, there were significant ($p < 0.05$) excess risks in the high-exposure categories of total THMs for ventricular septal defects [odds ratio (OR) = 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). **CONCLUSION:** In this large national study we found little evidence for a relationship between THM concentrations in drinking water and risk of congenital anomalies.

Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England.

Toledano M. B., Nieuwenhuijsen M. J., Best N., Whitaker H., Hambly P., de H. C., Fawell J., Jarup L. and Elliott P.
Environmental Health Perspectives 2005 113(2):225-32.

We investigated the association between total trihalomethanes (TTHMs) and risk of stillbirth and low and very low birth weight in three water regions in England, 1992-1998; associations with individual trihalomethanes (THMs) were also examined. Modeled estimates of quarterly TTHM concentrations in water zones, categorized as low (< 30 microg/L), medium (30-59 microg/L), or high (> or = 60 microg/L), were linked to approximately 1 million routine birth and stillbirth records using maternal residence at time of birth. In one region, where there was a positive socioeconomic deprivation gradient across exposure categories, there was also a positive, significant association of TTHM with risk of stillbirth and low and very low birth weight. Overall summary estimates across the three regions using a random-effects model to allow for between-region heterogeneity in exposure effects showed small excess risks in areas with high TTHM concentrations for stillbirths [odds ratio (OR) = 1.11; 95% confidence interval (CI), 1.00-1.23], low birth weight (OR = 1.09; 95% CI, 0.93-1.27), and very low birth weight (OR = 1.05; 95% CI, 0.82-1.34). Among the individual THMs, chloroform showed a similar pattern of risk as TTHM, but no association was found with concentrations of bromodichloromethane or total brominated THMs. Our findings overall suggest a significant association of stillbirths with maternal residence in areas with high TTHM exposure. Further work is needed looking at cause-specific stillbirths and effects of other disinfection by-products and to help differentiate between alternative (noncausal) explanations and those that may derive from the water supply.

Trihalomethanes in drinking water and spontaneous abortion.

Waller K., Swan S. H., DeLorenze G. and Hopkins B.
Epidemiology 1998 9(2):134-40.

Trihalomethanes (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) are common contaminants of chlorinated drinking water. Although animal data indicate that these compounds may be reproductive toxicants, little information exists on their relation to spontaneous abortion in humans. We examined exposure to trihalomethanes and spontaneous abortion in a prospective study of 5,144 pregnant women in a prepaid health plan. Seventy-eight drinking water utilities provided concurrent trihalomethane sampling data. We calculated total trihalomethane levels by averaging all measurements taken by the subject's utility during her first trimester. We calculated exposures to individual trihalomethanes in an analogous manner. Women who drank > or = 5 glasses per day of cold tapwater containing > or = 75 micrograms per liter total trihalomethanes had an adjusted odds ratio (OR) of 1.8 for spontaneous abortion [95% confidence interval (CI) = 1.1-3.0]. Of the four individual trihalomethanes, only high bromodichloromethane exposure (consumption of > or = 5 glasses per day of cold tapwater containing > or = 18 micrograms per liter bromodichloromethane) was associated with spontaneous abortion both alone (adjusted OR = 2.0; 95% CI = 1.2-3.5) and after adjustment for the other trihalomethanes (adjusted OR = 3.0; 95% CI = 1.4-6.6).

B.5 Chloroform

Relation between trihalomethane compounds and birth defects.

Dodds L. and King W. D.

Occupational and Environmental Medicine 2001 58(7):443-6.

OBJECTIVES: To evaluate the risk of birth defects relative to exposure to specific trihalomethanes in public water supplies. **METHODS:** A retrospective cohort study was conducted based on data from a population based perinatal database in Nova Scotia, Canada and from the results of routine water monitoring tests. The cohort consisted of women who had a singleton birth in Nova Scotia between 1988 and 1995 and who lived in an area with a municipal water supply. The birth defects analyzed included neural tube defects, cardiovascular defects, cleft defects, and chromosomal abnormalities. Two of the four trihalomethane compounds occur in large enough concentrations to be analyzed (chloroform and bromodichloromethane (BDCM)). **RESULTS:** Exposure to BDCM at concentrations of 20 microg/l or over was associated with an increased risk of neural tube defects (adjusted relative risk (RR) 2.5, 95% confidence interval (95% CI) 1.2 to 5.1) whereas exposure to chloroform was not. Exposure to BDCM of 20 microg/l and over was associated with decreased risks of cardiovascular anomalies (RR 0.3, 95% CI 0.2 to 0.7). There was a suggestion of an increased risk of chromosomal abnormalities associated with exposure to chloroform, and no evidence of any association between either trihalomethane compound and cleft defects. **CONCLUSIONS:** In this cohort, differences were found in the RR associated with exposure to chloroform and BDCM for each of the congenital anomalies under study. These findings point to the importance of examining specific byproduct compounds relative to risk for these birth outcomes and in particular implicate BDCM and other correlated disinfection byproducts in the aetiology of neural tube defects.

Dental workplace exposure and effect on fertility.

Dahl J. E., Sundby J., Hensten-Pettersen A. and Jacobsen N.

Scandinavian Journal of Work, Environment and Health 1999 25(3):285-90.

OBJECTIVES: This study assessed occupational exposure in dental surgeries on the basis of the reported use of dental materials and techniques and applied waiting-time-to-pregnancy methodology to study fertility in relation to the occupational exposure. **METHODS:** Data were collected retrospectively using a self-administered postal questionnaire addressing the occupational and reproductive history of the participants. The study groups consisted of 558 female dental surgeons and 450 high school teachers that had given birth in Norway to at least 1 living child. The present study comprised data from a total of 1408 pregnancies. The effects of practicing dentistry and of the given workplace exposure on fertility were analyzed with the discrete proportional hazard regression method. **RESULTS:** Most of the female dental surgeons were using amalgam for fillings during the period they tried to conceive, and 1/3 placed more than 50 fillings a week. Tooth-colored fillings were in limited use. Prior to 75% of the pregnancies, the dental surgeons reported handling chloroform-based root canal sealers. Forty percent of the dental surgeons were daily exposed to disinfectants containing ethanol and

benzene. No difference was found in fertility between the dental surgeons and the high school teachers. Exposure to mercury, chloroform, and benzene was not associated with decreased fertility, except for a possible effect of mercury in the last pregnancy of multiparous dental surgeons. CONCLUSIONS: Occupational exposures had no clear adverse effects on fertility among the female dental surgeons studied.

Trihalomethanes in drinking water and spontaneous abortion.

Waller K., Swan S. H., DeLorenze G. and Hopkins B.
Epidemiology 1998 9(2):134-40.

Trihalomethanes (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) are common contaminants of chlorinated drinking water. Although animal data indicate that these compounds may be reproductive toxicants, little information exists on their relation to spontaneous abortion in humans. We examined exposure to trihalomethanes and spontaneous abortion in a prospective study of 5,144 pregnant women in a prepaid health plan. Seventy-eight drinking water utilities provided concurrent trihalomethane sampling data. We calculated total trihalomethane levels by averaging all measurements taken by the subject's utility during her first trimester. We calculated exposures to individual trihalomethanes in an analogous manner. Women who drank ≥ 5 glasses per day of cold tapwater containing ≥ 75 micrograms per liter total trihalomethanes had an adjusted odds ratio (OR) of 1.8 for spontaneous abortion [95% confidence interval (CI) = 1.1-3.0]. Of the four individual trihalomethanes, only high bromodichloromethane exposure (consumption of ≥ 5 glasses per day of cold tapwater containing ≥ 18 micrograms per liter bromodichloromethane) was associated with spontaneous abortion both alone (adjusted OR = 2.0; 95% CI = 1.2-3.5) and after adjustment for the other trihalomethanes (adjusted OR = 3.0; 95% CI = 1.4-6.6).

C. Related articles and meeting abstracts

C.1 Total Trihalomethanes

Exposure assessment in epidemiologic studies of adverse pregnancy outcomes and disinfection byproducts.

King W. D., Dodds L., Armson B. A., Allen A. C., Fell D. B. and Nimrod C.
Journal of Exposure Analysis and Environmental Epidemiology 2004 14(6):466-72.

A major challenge in studies that examine the association between disinfection byproducts in drinking water and pregnancy outcomes is the accurate representation of a subject's exposure. We used household water samples and questionnaire information on water-use behavior to examine several aspects of exposure assessment: (i) the distribution and correlation of specific disinfection byproducts, (ii) spatial distribution system and temporal variation in byproduct levels, and (iii) the contribution of individual water-use behavior. The level of specific trihalomethanes (THMs) and haloacetic acids (HAAs) was determined for 360 household water samples in Eastern Ontario and Nova Scotia. Subjects were interviewed regarding tap water

ingestion and showering and bathing practices. In both provinces, total THMs correlated highly with chloroform (correlation coefficient (r) >0.95) and less so with total HAAs ($r = 0.74$ in Nova Scotia and $r = 0.52$ in Ontario). The correlation between total THMs and bromodichloromethane was high in Nova Scotia ($r = 0.63$), but low in Ontario ($r = 0.26$). The correlation was between THM level in individual household samples, and the mean THM level during the same time period from several distribution system samples was 0.63, while a higher correlation in THM level was observed for samples taken at the same location 1 year apart ($r = 0.87$). A correlation of 0.73 was found between household THM level and a total exposure measure incorporating ingestion, showering, and bathing behaviors. These results point to the importance of: measurement of different classes of byproducts; household rather than distribution system sampling; and, incorporation of subject behaviors in exposure assessment in epidemiologic studies of disinfection byproducts and adverse pregnancy outcomes.

Health risk to fetuses, infants and children from stage 1 disinfectants and disinfectant by-products (D/DBPs).

Bathija A. T.

Toxicologist. 2003 72(S-1):28.

The Safe Drinking Water Act (SDWA) of 1996, mandates EPA to consider the effects of contaminants in drinking water on the general population and on sensitive sub-groups such as infants, children, pregnant women and the elderly. Under the Executive Order 13045 of April 21, 1997, the Federal Health and Safety Standards must include an evaluation of potential risks to children from microbes and D/DBPs. Before the D/DBP rule was finalized in December 1998, a report was developed on The Health Risk to Fetuses, Infants and Children. This report considered the following issues: whether the D/DBPs caused developmental or reproductive effects; for carcinogenic D/DBPs, are children more likely to be affected than adults; and for noncarcinogenic effects, are children more sensitive than the adults. To answer these questions we evaluated toxicology studies, developmental and reproductive studies, and carcinogenicity and other systemic toxicity studies for the D/DBPs. We also evaluated epidemiology studies for carcinogenicity and developmental and reproductive effects. For Chloroform, Bromodichloromethane, Bromoform, Dichloroacetic Acid and Bromate the MCLG of zero for carcinogenic effects is protective of children's health. The Chloramine NOAEL/LOAEL is lower than NOAEL/LOAEL for developmental effects. Chlorine Dioxide and Chlorite MCLGs are based on developmental toxicity. The MCLGs for Chlorine and Dibromochloromethane are based on other non-carcinogenic effects. Based on our evaluations, it was concluded that for the Stage 1 D/DBPs the MCLGs are "protective" for developmental and reproductive effects.

Exposures to drinking water chlorination by-products in a Russian city.

Egorov A. I., Tereschenko A. A., Altshul L. M., Vartiainen T., Samsonov D., LaBrecque B., Mäki-Paakkanen J., Drizhd N. L. and Ford T. E.

International Journal of Hygiene and Environmental Health 2003 206(6):539-51.

Exposures to water disinfection by-products (DBPs) via ingestion of drinking water, and dermal absorption and inhalation during showering/bathing were assessed in the city of Cherepovets,

Russia, which uses heavy chlorination to disinfect organic-rich surface water. Concentrations of DBPs (mean +/- standard deviation) in tap water were the following: total trihalomethanes (THMs) 205 +/- 70 micrograms/l, five haloacetic acids (HAAs) 150 +/- 30 micrograms/l, and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (mutagen X or MX) 160 +/- 50 ng/l. Concentrations of THMs and HAAs exceeded the corresponding US standards by a factor of 2.5, while MX concentrations were the highest ever reported. The mutagenic activity of tap water extracts in the Salmonella TA-100 assay was 14,900 net revertants/l. Concentrations of chloroform in breathing zone air in bathrooms during showering were 330 +/- 260 micrograms/m³, shower room air at an industrial plant 2,600 +/- 1,100 micrograms/m³, and bedrooms of local residents 2 +/- 2 micrograms/m³. The mean concentration of chloroform was 3.2 micrograms/m³ in exhaled air samples collected before showering and 110 micrograms/m³ after showering. Data on water ingestion and water use practices in the general population and for pregnant women were collected using questionnaires and diaries. Due to concerns over microbiological safety of water, average daily consumption of non-boiled tap water in pregnant women was only 0.01 l/day, while consumption of boiled tap water was 0.81 l/day. This resulted in low ingestion exposures to volatile THMs. Inhalation and dermal absorption determined total exposures to these compounds. HAAs and MX persist in boiled water and drinks resulting in high ingestion exposures. Several brands of inexpensive home water filters were tested for removal of these compounds. To demonstrate a method of exposure reduction in a sensitive subpopulation, the most efficient filters were given to a group of pregnant women. These women and a control group of pregnant women without filters maintained water ingestion diaries for two weeks. The use of home filters resulted in reduction of exposures to HAAs by a factor of three and a greater reduction in exposures to MX.

Chlorination Disinfection By-Products And Adverse Birth Outcomes: A Retrospective Cohort Study In Three Regions In England.

Toledano M. B., Nieuwenhuijsen M. J., Best N. G., Whitaker H., De H. C., Cobley N., Fawell J. and Elliott P.
Epidemiology. 2003 14(5 Suppl).

Recent epidemiological studies of the relationship between disinfection by-products (DBPs), from chlorination, in public drinking water supply and adverse birth outcomes, have reported inconsistent and inconclusive findings. We report here on the largest study to date to examine the relationship of total trihalomethanes (TTHMs) and the individual trihalomethanes (THMs) to birth weight and still birth prevalence, between 1993 and 1998, for regions covered by three water companies in England. Initial results from this ongoing study using modelled annual TTHM estimates were found to differ between each of the three water supply areas. There was some evidence of confounding by social deprivation and ethnicity, which was further explored by analysis of information on the lifestyle characteristics of all women of reproductive age in the study regions from National survey data. Here, we report on the next stage of the study using modelled quarterly (3 monthly) estimates of the individual THMs in water zones. We modelled THM measurements using a Bayesian hierarchical mixture model, taking into account heterogeneity in THM levels between water originating from different source types (e.g. ground, lowland surface or upland surface), quarterly variation in THM levels and uncertainty in the true

value of undetected and rounded measurements. These modelled estimates were linked using Geographical Information Systems to routine birth and stillbirth records based on location of maternal residence at the time of birth to obtain a weighted third trimester exposure estimate for each birth. Exposure categories were constructed as follows: for chloroform (low (< 20 mg/1), medium (20-40 mg/1) and high (> 40 mg/1), bromodichloromethane (low (< 6 mg/1), medium (6-12 mg/1) and high (> 12 mg/1), dibromochloromethane (low (After exclusion of multiple births and births from water zones without valid THM data, we studied a total of 934,843 live and stillbirths. Exposure-response relationships were explored using multiple logistic regression analysis with weighted third trimester individual THM estimates and adjustment for e.g. gender, maternal age and deprivation. Analysis of the individual THMs, as opposed to TTHMs, is essential to characterise more precisely the effects of complex mixtures of DBPs on the developing foetus, and refine our understanding of this important area of research.

Constructing scientific authorities: issue framing of chlorinated disinfection byproducts in public health.

Driedger S. M., Eyles J., Elliott S. D. and Cole D. C.
Risk Analysis 2002 22(4):789-802.

The practice of chlorine disinfection of drinking water to reduce microbial risks provides substantial benefits to public health. However, increasing concern around potential risks of cancer associated with exposure to chlorinated disinfection byproducts confuses this issue. This article examines the science agenda regarding chlorinated disinfection byproducts (CDBP) and cancer in Canada and the United States, focusing on the social construction of scientific knowledge claims and evidence. Data for this analysis were obtained from published documents as well as from in-depth interviews with epidemiologists and toxicologists centrally involved with the issue in both countries. Results of the analysis suggest that toxicological scientists want to close the door on the "chloroform issue" due to increasing evidence that chloroform is safe at low doses, because epidemiological scientists can no longer move forward the cancer science until significant improvements can be made in assessing human exposures, and because the scientific foci of research on DBP have shifted accordingly. Further, a distinction emerges in terms of how scientific uncertainties are interpreted when they cross-cut disciplines in the context of human health risk assessment. We suggest this tension reflects a balance of how uncertainty and authorities are managed in a mandated science-policy domain. Sufficient evidence was provided to keep the DBP issue on the regulatory agenda and to generate additional research, yet authorities and concomitant interpretations of uncertainty were contested. Such science generation and contestation inevitably influences complex risk assessment processes with respect to what water-related health risks are addressed and how.

Use of routinely collected data on trihalomethane in drinking water for epidemiological purposes.

Keegan T., Whitaker H., Nieuwenhuijsen M. J., Toledano M. B., Elliott P., Fawell J., Wilkinson M. and Best N.

Occupational and Environmental Medicine 2001 58(7):447-52.

OBJECTIVES: To explore the use of routinely collected trihalomethane (THM) measurements for epidemiological studies. Recently there has been interest in the relation between byproducts of disinfection of public drinking water and certain adverse reproductive outcomes, including stillbirth, congenital malformations, and low birth weight. **METHOD:** Five years of THM readings (1992--6), collected for compliance with statutory limits, were analysed. One water company in the north west of England, divided into 288 water zones, provided 15,984 observations for statistical analysis. On average each zone was sampled 11.1 times a year. Five year, annual, monthly, and seasonal variation in THMs were examined as well as the variability within and between zones. **RESULTS:** Between 1992 and 1996 the total THM (TTHM) annual zone means were less than half the statutory concentration, at approximately 46 microg/l. Differences in annual water zone means were within 7%. Over the study period, the maximum water zone mean fell from 142.2 to 88.1 microg/l. Mean annual concentrations for individual THMs (microg/l) were 36.6, 8.0, and 2.8 for chloroform, bromodichloromethane (BDCM), and dibromochloromethane (DBCM) respectively. Bromoform data were not analysed, because a high proportion of the data were below the detection limit. The correlation between chloroform and TTHM was 0.98, between BDCM and TTHM 0.62, and between DBCM and TTHM -0.09. Between zone variation was larger than within zone variation for chloroform and BDCM, but not for DBCM. There was only little seasonal variation (< 3%). Monthly variation was found although there were no consistent trends within years. **CONCLUSION:** In an area where the TTHM concentrations were less than half the statutory limit (48 microg/l) chloroform formed a high proportion of TTHM. The results of the correlation analysis suggest that TTHM concentrations provided a good indication of chloroform concentrations, a reasonable indication of BDCM concentrations, but no indication of DBCM. Zone means were similar over the years, but the maximum concentrations reduced considerably, which suggests that successful improvements in treatment have been made to reduce high TTHM concentrations in the area. For chloroform and BDCM, the main THMs, the component between water zones was greater than variation within water zones and explained most of the overall exposure variation. Variation between months and seasons was low and showed no clear trends within years. The results indicate that routinely collected data can be used to obtain exposure estimates for epidemiological studies at a small area level.

Assessing exposure to disinfection by-products in women of reproductive age living in Corpus Christi, Texas, and Cobb county, Georgia: descriptive results and methods.

Lynberg M., Nuckols J. R., Langlois P., Ashley D., Singer P., Mendola P., Wilkes C., Krapfl H., Miles E., Speight V., Lin B., Small L., Miles A., Bonin M., Zeitz P., Tadmok A., Henry J. and Forrester M. B.

Environmental Health Perspectives 2001 109(6):597-604.

We conducted a field study in Corpus Christi, Texas, and Cobb County, Georgia, to evaluate exposure measures for disinfection by-products, with special emphasis on trihalomethanes (THMs). Participants were mothers living in either geographic area who had given birth to healthy infants from June 1998 through May 1999. We assessed exposure by sampling blood and water and obtaining information about water use habits and tap water characteristics. Two 10-mL whole blood samples were collected from each participant before and immediately after her shower. Levels of individual THM species (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) were measured in whole blood [parts per trillion (pptr)] and in water samples (parts per billion). In the Corpus Christi water samples, brominated compounds accounted for 71% of the total THM concentration by weight; in Cobb County, chloroform accounted for 88%. Significant differences in blood THM levels were observed between study locations. For example, the median baseline blood level of bromoform was 0.3 pptr and 3.5 pptr for participants in Cobb County and Corpus Christi, respectively ($p = 0.0001$). Differences were most striking in blood obtained after showering. For bromoform, the median blood levels were 0.5 pptr and 17 pptr for participants in Cobb County and Corpus Christi, respectively ($p = 0.0001$). These results suggest that blood levels of THM species vary substantially across populations, depending on both water quality characteristics and water use activities. Such variation has important implications for epidemiologic studies of the potential health effects of disinfection by-products.

Overview: developmental risks of disinfectant by-products symposium.

Hunter S.

Teratology 2000 61(6):464.

Clean and safe drinking water is a fundamental requirement for everyone. Clearly, the disinfection of drinking water has led to a dramatic reduction in water-related illnesses. However, recent epidemiological studies have heightened concern over adverse pregnancy outcomes and their association to consumption of disinfected water. This symposium will discuss the potential for disinfectant by-products (DBFs) and other drinking water contaminants to alter pregnancy outcome or embryonic/fetal development. The speakers will provide information ranging from disinfection processes, epidemiological studies of pregnancy outcome in women drinking disinfected water, and the effects of drinking water contaminants in animal studies. Mr. Stuart Krasner, Metropolitan Water District of Southern California, will begin the symposium with a discussion of the disinfection process and the types of disinfection by-products (DBPs) that are produced. Differences in the source water to be disinfected as well as the disinfection method influence the types and concentrations of DBFs produced, which in turn influence the association between DBFs and pregnancy outcome evaluated in epidemiological

studies. Dr. John Reif, Colorado State University, will then review the epidemiological studies reporting associations between drinking water "quality" and pregnancy outcome. Several studies indicate that drinking water with the highest levels of trihalomethanes (THMs) are associated with a variety of adverse outcomes, including increased spontaneous abortions, decreased birth weight and increased neural tube and heart defects. Dr. Michael Narotsky, US Environmental Protection Agency, will be the first of several speakers discussing the effects of specific contaminants in animal models. He will focus on the effects of THMs, such as bromodichloromethane (BDCM), and their effects on pregnancy maintenance in rats. Studies to establish the mode of action for BDCM-induced full-litter loss and the possible relationship to spontaneous abortions observed in epidemiology studies will be discussed. Dr. Ornella Selmin, University of Arizona Health Sciences Center, will next talk about her work on induction of heart defects resulting from trichloroethylene exposure in animal models. Changes in expression of several molecules have been identified and can be used to identify effectors or serve as biomarkers of defects. Dr. Rochelle Tyl, Research Triangle Institute, will conclude the symposium with a review of the effects of DBFs in animal models. The relationship between doses that induce a toxic response in animals and the levels of human exposure will provide the context for a better understanding of the potential for DBFs to alter pregnancy outcome and embryonic/fetal development.

[Human exposure to trihalomethanes in drinking water].

Tominaga M. Y. and Midio A. F.

Revista de Saúde Pública 1999 33(4):413-21.

Halogenated hydrocarbon compounds, some of them recognized as carcinogenic to different animal species can be found in drinking water. Chloroform, bromodichloromethane, dibromochloromethane and bromoform are the most important trihalomethanes found in potable water. They are produced in natural waters during chlorinated disinfection by the halogenation of precursors, specially humic and fulvic compounds. The review, in the MEDLINE covers the period from 1974 to 1998, presents the general aspects of the formation of trihalomethanes, sources of human exposure and their toxicological meaning for exposed organisms: toxicokinetic disposition and spectrum of toxic effects (carcinogenic, mutagenic and teratogenic).

Health risks to fetuses, infants, and children (Final Stage 1 D/DBP Rule).

Bathija A., Chiu N., Conerly O., Dellarco V., Khanna K., Manibusan M., Patel Y. and Schoeny R.

NTIS Technical Report 1998 1113790(46).

In developing the Final Stage 1 D/DBP Rule, risks to sensitive subpopulations including fetuses and children were taken into account in the assessments of D/DBPs. To determine whether fetuses and children are more sensitive than adults, the following issues were considered: Do D/DBPs cause developmental and reproductive effects at doses below those causing systemic toxicity or cancer? Are fetuses and children more susceptible than adults to cancer from D/DBPs? Are fetuses and children more susceptible than adults to the systemic toxicity of D/DBPs? This document evaluates the available data for each of the D/DBPs used for deriving

the MCLG or MRDLG, to determine if the derived MCLGs and/or MRDLGs are protective for the fetuses and children. Table 3 summarized the comparison of toxicity endpoints for the various D/DBPs. As can be seen in the table, chloroform, BDCM, bromoform, DCA, and bromate are considered to be probable human carcinogens. MCLGs of zero were selected after consideration of the potential carcinogenicity of the chemicals. This MCLG of zero would protect both children and adults. The MCLG/MRDLGs for DBCM, and chloramine were based on systemic toxicity. The NOAEL/LOAEL used to derive the numbers are lower than the NOEL/LOAELs for developmental effects; therefore, the MCLG/MRDLG would be protective of infants and children. In the case of chlorine, the MCLG is based on systemic toxicity because the NOAEL of 5 mg/kg/day based on developmental effects is the highest dose tested and is therefore not a true NOAEL. For chlorine dioxide, chlorite, and TCA, the MCLG/MRDLGs are calculated on data from developmental studies; hence the numbers derived would be protective for both children and adults. It can be concluded that the MCLG/MRDLGs of all the D/DBPs in the Stage 1 D/DBP Rule are protective of fetuses, infants, and children.

Trihalomethane levels in Madras public drinking water supply system and its impact on public health.

Rajan S., Azariah J. and Bauer U.

Zentralblatt für Hygiene und Umweltmedizin (International Journal of Hygiene and Environmental Medicine) 1990 189(4):312-32.

It is known that trihalomethanes (THM) are formed during chlorination of drinking water for disinfection. Heightened concern about these substances is due to the fact that THMs are now characterized as potential mutagen, carcinogen and teratogen. Thus, it is a risk factor in human beings. In the present study, a total number of 13 stations located in different drinking water trunk mains of the city of Madras were analysed for THM using the Gas Liquid Chromatographic method. It is reported that THM are formed after treatment of raw water with chlorine at the levels required for disinfection. The THM level in drinking water increased towards the dead-end of the water trunk mains. A relationship between the distance travelled by the potable water and the level of THM was established. At certain stations, the total trihalomethanes level (TTHM) was found to exceed the EPA's maximum contaminant level. Further, an intermittent addition of the precursors for the formation of THM through the seepage of polluted River Cooum water into the pipe lines has been demonstrated. An experiment on the trihalomethane formation potential (THMFP) clearly revealed the occurrence of higher magnitude of humic substances in source water. Therefore, it is suggested that if suitable steps are not taken, various environmental factors may trigger the THM kinetics. Hence, it is obvious that pretreatment regulations proposed by developed countries are essential if safe drinking water is to be supplied to the people of Madras.

C.2 BDCM

Bromodichloromethane inhibits human placental trophoblast differentiation.

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. and Douglas G. C.
Toxicological Sciences 2004 78(1):166-74.

Epidemiological data suggest an association between exposures to bromodichloromethane (BDCM), a trihalomethane found in drinking water as a result of drinking water disinfection, and an increased risk of spontaneous abortion. We previously hypothesized that BDCM targets the placenta and showed that the secretion of chorionic gonadotrophin (CG) was reduced in primary cultures of human term syncytiotrophoblasts exposed to BDCM. In the present study we extend this observation by evaluating the effects of BDCM on the morphological differentiation of mononucleated cytotrophoblast cells to multinucleated syncytiotrophoblast-like colonies. Addition of BDCM to cytotrophoblast cultures inhibited the subsequent formation of multinucleated colonies in a dose-dependent manner, as determined by immunocytochemical staining for desmosomes and nuclei. The effect was seen at BDCM concentrations between 0.02 and 2 mM and was confirmed by quantitative image analysis. Secretion of bioactive and immunoreactive chorionic gonadotropin was also significantly inhibited in a dose-dependent manner under these culture conditions, and cellular levels of CG were also reduced. Trophoblast viability was not compromised by exposure to BDCM. We conclude that BDCM disrupts syncytiotrophoblast formation and inhibits CG secretion in vitro. Although other tissue targets are not ruled out, these data substantiate the idea that BDCM targets the placenta and could have implications for understanding the adverse pregnancy outcomes associated with BDCM exposure in humans.

Effect of bromodichloromethane on chorionic gonadotrophin secretion by human placental trophoblast cultures.

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. and Lasley B. L.
Toxicological Sciences 2003 76(1):75-82.

Bromodichloromethane (BDCM) is a trihalomethane found in drinking water as a by-product of disinfection processes. BDCM is hepatotoxic and nephrotoxic in rodents and has been reported to cause strain-specific full-litter resorption in F344 rats during the luteinizing hormone-dependent phase of pregnancy. In humans, epidemiological studies suggest an association between exposure to BDCM in drinking water and increased risk of spontaneous abortion. To begin to address the mechanism(s) of BDCM-induced spontaneous abortion, we hypothesized that BDCM targets the placenta. Primary cultures of human term trophoblast cells were used as an in vitro model to test this hypothesis. Trophoblasts were allowed to differentiate into multinucleated syncytiotrophoblast-like colonies, after which they were incubated for 24 h with different concentrations of BDCM (20 nM to 2 mM). Culture media were collected and assayed for immunoreactive and bioactive chorionic gonadotropin (CG). Cultures exposed to BDCM showed a dose-dependent decrease in the secretion of immunoreactive CG as well as bioactive

CG. The lowest effective BDCM concentration was 20 nM, approximately 35-times higher than the maximum concentration reported in human blood (0.57 nM). Trophoblast morphology and viability were similar in controls and cultures exposed to BDCM. We conclude that BDCM perturbs CG secretion by differentiated trophoblasts in vitro. This suggests that the placenta is a likely target of BDCM toxicity in the human and that this could be related to the adverse pregnancy outcomes associated with BDCM.

Use of routinely collected data on trihalomethane in drinking water for epidemiological purposes.

Keegan T., Whitaker H., Nieuwenhuijsen M. J., Toledano M. B., Elliott P., Fawell J., Wilkinson M. and Best N.

Occupational and Environmental Medicine 2001 58(7):447-52.

OBJECTIVES: To explore the use of routinely collected trihalomethane (THM) measurements for epidemiological studies. Recently there has been interest in the relation between byproducts of disinfection of public drinking water and certain adverse reproductive outcomes, including stillbirth, congenital malformations, and low birth weight. **METHOD:** Five years of THM readings (1992--6), collected for compliance with statutory limits, were analysed. One water company in the north west of England, divided into 288 water zones, provided 15,984 observations for statistical analysis. On average each zone was sampled 11.1 times a year. Five year, annual, monthly, and seasonal variation in THMs were examined as well as the variability within and between zones. **RESULTS:** Between 1992 and 1996 the total THM (TTHM) annual zone means were less than half the statutory concentration, at approximately 46 microg/l. Differences in annual water zone means were within 7%. Over the study period, the maximum water zone mean fell from 142.2 to 88.1 microg/l. Mean annual concentrations for individual THMs (microg/l) were 36.6, 8.0, and 2.8 for chloroform, bromodichloromethane (BDCM), and dibromochloromethane (DBCM) respectively. Bromoform data were not analysed, because a high proportion of the data were below the detection limit. The correlation between chloroform and TTHM was 0.98, between BDCM and TTHM 0.62, and between DBCM and TTHM -0.09. Between zone variation was larger than within zone variation for chloroform and BDCM, but not for DBCM. There was only little seasonal variation (< 3%). Monthly variation was found although there were no consistent trends within years. **CONCLUSION:** In an area where the TTHM concentrations were less than half the statutory limit (48 microg/l) chloroform formed a high proportion of TTHM. The results of the correlation analysis suggest that TTHM concentrations provided a good indication of chloroform concentrations, a reasonable indication of BDCM concentrations, but no indication of DBCM. Zone means were similar over the years, but the maximum concentrations reduced considerably, which suggests that successful improvements in treatment have been made to reduce high TTHM concentrations in the area. For chloroform and BDCM, the main THMs, the component between water zones was greater than variation within water zones and explained most of the overall exposure variation. Variation between months and seasons was low and showed no clear trends within years. The results indicate that routinely collected data can be used to obtain exposure estimates for epidemiological studies at a small area level.

Overview: developmental risks of disinfectant by-products symposium.

Hunter S.

Teratology 2000 61(6):464.

Clean and safe drinking water is a fundamental requirement for everyone. Clearly, the disinfection of drinking water has led to a dramatic reduction in water-related illnesses. However, recent epidemiological studies have heightened concern over adverse pregnancy outcomes and their association to consumption of disinfected water. This symposium will discuss the potential for disinfectant by-products (DBFs) and other drinking water contaminants to alter pregnancy outcome or embryonic/fetal development. The speakers will provide information ranging from disinfection processes, epidemiological studies of pregnancy outcome in women drinking disinfected water, and the effects of drinking water contaminants in animal studies. Mr. Stuart Krasner, Metropolitan Water District of Southern California, will begin the symposium with a discussion of the disinfection process and the types of disinfection by-products (DBPs) that are produced. Differences in the source water to be disinfected as well as the disinfection method influence the types and concentrations of DBFs produced, which in turn influence the association between DBFs and pregnancy outcome evaluated in epidemiological studies. Dr. John Reif, Colorado State University, will then review the epidemiological studies reporting associations between drinking water "quality" and pregnancy outcome. Several studies indicate that drinking water with the highest levels of trihalomethanes (THMs) are associated with a variety of adverse outcomes, including increased spontaneous abortions, decreased birth weight and increased neural tube and heart defects. Dr. Michael Narotsky, US Environmental Protection Agency, will be the first of several speakers discussing the effects of specific contaminants in animal models. He will focus on the effects of THMs, such as bromodichloromethane (BDCM), and their effects on pregnancy maintenance in rats. Studies to establish the mode of action for BDCM-induced full-litter loss and the possible relationship to spontaneous abortions observed in epidemiology studies will be discussed. Dr. Ornella Selmin, University of Arizona Health Sciences Center, will next talk about her work on induction of heart defects resulting from trichloroethylene exposure in animal models. Changes in expression of several molecules have been identified and can be used to identify effectors or serve as biomarkers of defects. Dr. Rochelle Tyl, Research Triangle Institute, will conclude the symposium with a review of the effects of DBFs in animal models. The relationship between doses that induce a toxic response in animals and the levels of human exposure will provide the context for a better understanding of the potential for DBFs to alter pregnancy outcome and embryonic/fetal development.

C.3 DBCM

Use of routinely collected data on trihalomethane in drinking water for epidemiological purposes.

Keegan T., Whitaker H., Nieuwenhuijsen M. J., Toledano M. B., Elliott P., Fawell J., Wilkinson M. and Best N.

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C.4 Bromoform

No abstracts were identified in this category.

C.5 Chloroform

The relationship between water concentrations and individual uptake of chloroform: a simulation study.

Whitaker H. J., Nieuwenhuijsen M. J. and Best N. G.
Environmental Health Perspectives 2003 111(5):688-94.

We simulated the relationship between water chloroform concentrations and chloroform uptake in pregnant women to assess the potential extent of exposure measurement error in epidemiologic studies of the health effects of exposure to water disinfection by-products. Data from the literature were used to assign statistical distributions to swimming pool chloroform concentrations, frequency and duration of swimming, showering and bathing, and average tap water consumption. Measured increases in blood chloroform concentrations after these activities were used to estimate average uptake per microgram per liter chloroform in the water, per minute spent in the activity or per liter consumed. Given average tap water chloroform concentrations from a U.K. epidemiologic study, an average daily uptake over 90 days was simulated for 300,000 mothers. The correlation between simulated uptakes and home tap chloroform concentration was 0.6. Mothers who swam regularly received far greater doses than did nonswimmers. Results suggest there will be considerable attenuation in risk estimates and/or power loss in epidemiologic studies if the putative agent is chloroform.

Constructing scientific authorities: issue framing of chlorinated disinfection byproducts in public health.

Driedger S. M., Eyles J., Elliott S. D. and Cole D. C.
Risk Analysis 2002 22(4):789-802.

The practice of chlorine disinfection of drinking water to reduce microbial risks provides substantial benefits to public health. However, increasing concern around potential risks of cancer associated with exposure to chlorinated disinfection byproducts confuses this issue. This article examines the science agenda regarding chlorinated disinfection byproducts (CDBP) and cancer in Canada and the United States, focusing on the social construction of scientific knowledge claims and evidence. Data for this analysis were obtained from published documents as well as from in-depth interviews with epidemiologists and toxicologists centrally involved with the issue in both countries. Results of the analysis suggest that toxicological scientists want to close the door on the "chloroform issue" due to increasing evidence that chloroform is safe at low doses, because epidemiological scientists can no longer move forward the cancer science until significant improvements can be made in assessing human exposures, and because the scientific foci of research on DBP have shifted accordingly. Further, a distinction emerges in terms of how scientific uncertainties are interpreted when they cross-cut disciplines in the

context of human health risk assessment. We suggest this tension reflects a balance of how uncertainty and authorities are managed in a mandated science-policy domain. Sufficient evidence was provided to keep the DBP issue on the regulatory agenda and to generate additional research, yet authorities and concomitant interpretations of uncertainty were contested. Such science generation and contestation inevitably influences complex risk assessment processes with respect to what water-related health risks are addressed and how.

Use of routinely collected data on trihalomethane in drinking water for epidemiological purposes.

Keegan T., Whitaker H., Nieuwenhuijsen M. J., Toledano M. B., Elliott P., Fawell J., Wilkinson M. and Best N.

Occupational and Environmental Medicine 2001 58(7):447-52.

OBJECTIVES: To explore the use of routinely collected trihalomethane (THM) measurements for epidemiological studies. Recently there has been interest in the relation between byproducts of disinfection of public drinking water and certain adverse reproductive outcomes, including stillbirth, congenital malformations, and low birth weight. **METHOD:** Five years of THM readings (1992--6), collected for compliance with statutory limits, were analysed. One water company in the north west of England, divided into 288 water zones, provided 15,984 observations for statistical analysis. On average each zone was sampled 11.1 times a year. Five year, annual, monthly, and seasonal variation in THMs were examined as well as the variability within and between zones. **RESULTS:** Between 1992 and 1996 the total THM (TTHM) annual zone means were less than half the statutory concentration, at approximately 46 microg/l. Differences in annual water zone means were within 7%. Over the study period, the maximum water zone mean fell from 142.2 to 88.1 microg/l. Mean annual concentrations for individual THMs (microg/l) were 36.6, 8.0, and 2.8 for chloroform, bromodichloromethane (BDCM), and dibromochloromethane (DBCM) respectively. Bromoform data were not analysed, because a high proportion of the data were below the detection limit. The correlation between chloroform and TTHM was 0.98, between BDCM and TTHM 0.62, and between DBCM and TTHM -0.09. Between zone variation was larger than within zone variation for chloroform and BDCM, but not for DBCM. There was only little seasonal variation (< 3%). Monthly variation was found although there were no consistent trends within years. **CONCLUSION:** In an area where the TTHM concentrations were less than half the statutory limit (48 microg/l) chloroform formed a high proportion of TTHM. The results of the correlation analysis suggest that TTHM concentrations provided a good indication of chloroform concentrations, a reasonable indication of BDCM concentrations, but no indication of DBCM. Zone means were similar over the years, but the maximum concentrations reduced considerably, which suggests that successful improvements in treatment have been made to reduce high TTHM concentrations in the area. For chloroform and BDCM, the main THMs, the component between water zones was greater than variation within water zones and explained most of the overall exposure variation. Variation between months and seasons was low and showed no clear trends within years. The results indicate that routinely collected data can be used to obtain exposure estimates for epidemiological studies at a small area level.

Chloroform: a review of its metabolism, teratogenic, mutagenic, and carcinogenic potential.

Davidson I. W., Sumner D. D. and Parker J. C.
Drug and Chemical Toxicology 1982 5(1):1-87.

There is no available abstract for this reference.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

A.1 Total Trihalomethanes

In vitro assessment of the effect of halogenated hydrocarbons: chloroform, dichloromethane, and dibromoethane on embryonic development of the rat.

Brown-Woodman P. D., Hayes L. C., Huq F., Herlihy C., Picker K. and Webster W. S. *Teratology* 1998 57(6):321-33.

Halogenated hydrocarbons are widely used in industry, the laboratory, and in the home. In the present study three of these solvents--chloroform, dichloromethane, and dibromoethane--were examined for embryotoxic/teratogenic potential using rat embryo culture. The results showed that each of the solvents had a concentration-dependent embryotoxic effect on the developing rat embryo in vitro. The effect and no-effect concentrations (expressed in $\mu\text{mol/ml}$ culture medium), respectively, for each of the halogenated hydrocarbons tested were: dibromoethane--0.33, of chloroform and dichloromethane found to be embryotoxic in the present study were compared to reported blood levels attained following controlled human exposure. In the industrial situation, if the current exposure levels are adhered to, chloroform and dichloromethane appear to have little potential for reproductive toxicity in the human. Fatal or near fatal solvent levels would be required in the mother for the embryotoxic level to be reached. For dibromoethane, there are no reports following controlled human exposure presumably due to its carcinogenicity. In an attempt to elucidate the mechanism of embryotoxicity, histological studies were performed after exposure of rat embryos to an embryotoxic level of each of the halogenated hydrocarbons studied, for increasing time periods up to the standard 40-hour culture. Marked cell death in the neuroepithelium of the developing neural tube was a prominent feature in all embryos exposed to an embryotoxic level of these solvents for periods of 16 hours or longer.

A teratological assessment of four trihalomethanes in the rat.

Ruddick J. A., Villeneuve D. C., Chu I. and Valli V. E. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes* 1983 18(3):333-49.

Four trihalomethanes were administered by gavage to Sprague-Dawley rats from day 6 to day 15 of gestation. Chloroform (Ch) was administered at levels of 100, 200 and 400 mg/kg and bromoform (Br), bromodichloromethane (BDCM) and chlorodibromomethane (CDBM) were administered at levels of 50, 100 or 200 mg/kg/day. A separate control was used for each compound. Maternal weight gain was depressed in all groups receiving Ch and at the highest dose levels of BDCM and CDBM. Ch administration caused decreased maternal hemoglobin and hematocrit values at all dose levels and also produced increased serum inorganic phosphorus and cholesterol at the highest dose. Liver enlargement was observed at all dose levels of Ch but in no other treatment groups. Evidence of a fetotoxic response was observed with Ch, CDBM and Br

but not BDCM. No dose-related histopathological changes were observed in either mothers or fetuses as a result of treatment. None of the chemicals tested produced any teratogenic effects.

B.2 BDCM

Effects of bromodichloromethane on ex vivo and in vitro luteal function and bromodichloromethane tissue dosimetry in the pregnant F344 rat.

Bielmeier S. R., Murr A. S., Best D. S., Harrison R. A., Pegram R. A., Goldman J. M. and Narotsky M. G.

Toxicology In Vitro 2007 21(5):919-28.

Bromodichloromethane (BDCM), a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption, in F344 rats when treated during the luteinizing hormone (LH)-dependent period. This effect is associated with reduced maternal serum progesterone (P) and LH levels, suggesting that BDCM disrupts secretion of LH. To test the hypothesis that BDCM also affects luteal responsiveness to LH, we used ex vivo and in vitro approaches. For the ex vivo study (i.e., in vivo exposure followed by in vitro assessment), dams were dosed by gavage on gestation days (GD) 6-9 (plug day=GD 0) at 0 or 100 mg/kg/d. One hour after the GD-9 dose, rats were killed, blood was collected, and tissue concentrations of BDCM were assessed. Corpora lutea (CL) were incubated with or without hCG, an LH agonist, to stimulate P secretion. For the in vitro study, CL were pooled from untreated F344 rats on GD 9 and cultured with BDCM at 0, 0.01, 0.10 or 3.0 mM. BDCM was found at highest concentrations in adrenal, ovarian, adipose, and hypothalamic tissues. BDCM treatment decreased serum P and LH levels in vivo. Ex vivo, however, BDCM-exposed CL showed >2-fold increases in P secretion relative to controls. Both control and BDCM-exposed CL displayed a 2.4-fold increase in P secretion in response to hCG challenge. In contrast, in vitro exposures reduced CL responsiveness in a dose-related fashion while baseline levels were unaffected. It is unclear if the ex vivo 'rebound' reflects the removal of the CL from a possible direct inhibitory influence of BDCM, or a response to diminished LH stimulation in vivo. Thus, these data suggest that BDCM disrupts pregnancy in F344 rats via two modes: disruption of LH secretion, and disruption of the CL's ability to respond to LH.

Serum hormone characterization and exogenous hormone rescue of bromodichloromethane-induced pregnancy loss in the F344 rat.

Bielmeier S. R., Best D. S. and Narotsky M. G.

Toxicological Sciences 2004 77(1):101-8.

Previously, we demonstrated that bromodichloromethane (BDCM), a drinking water disinfection by-product, causes pregnancy loss in F344 rats when given on gestational days (GD) 6-10, encompassing the luteinizing hormone (LH)-dependent period of pregnancy (GD 7-10). Pregnancy loss, i.e., full-litter resorption, was associated with reduced serum progesterone levels; however, we were unable to identify an effect on serum LH. Here, we reevaluated serum LH levels using the more sensitive technique, DELFIA(R). We further sought to better define the temporal pattern of endocrine disruption caused by BDCM during pregnancy with more frequent

sampling. Lastly, we attempted to prevent BDCM-induced pregnancy loss using exogenous progesterone or human chorionic gonadotropin (hCG), an LH-agonist. BDCM, in 10% Alkamuls(R), was dosed at 75 mg/kg/day by gavage to F344 rats on GD 6-10 (plug day = GD 0). BDCM-induced pregnancy loss was associated with marked reductions in serum progesterone and LH on GD 10. The decrease in serum LH consistently preceded the decrease in progesterone. In the hormone replacement studies, BDCM and progesterone were administered on GD 6-10, hCG on GD 8-10. BDCM was delivered at 100 mg/kg/day, progesterone at 10 mg/kg twice daily, and hCG at 0.5 IU/0.2 ml/rat. Both progesterone and hCG prevented BDCM-induced pregnancy loss. Thus, BDCM-induced pregnancy loss was associated with marked GD-10 reductions in serum LH and corresponding decreases in progesterone. Furthermore, coadministration of an LH agonist prevented pregnancy loss, supporting the hypothesis that BDCM-induced pregnancy loss in the rat occurs via an LH-mediated mode of action.

Pregnancy loss in the rat caused by bromodichloromethane.

Bielmeier S. R., Best D. S., Guidici D. L. and Narotsky M. G.
Toxicological Sciences 2001 59(2):309-15.

Bromodichloromethane (BDCM), a trihalomethane, is a by-product of the chlorination of drinking water. In a recent epidemiological study, consumption of BDCM was associated with an increased risk of spontaneous abortion in pregnant women. We have previously shown that BDCM causes pregnancy loss, i.e., full-litter resorption (FLR), in the F344 rat. The mode of action was investigated, with three main findings. First, there was a dramatic difference in sensitivity between F344 and Sprague-Dawley (SD) rat strains. Following aqueous gavage treatment on gestational days (GD) 6-10, F344 rats had a 62% incidence of FLR at 75 mg/kg/day, whereas all SD rats maintained their litters. Second, the critical period encompassed the luteinizing hormone (LH)-dependent period of pregnancy. Rats treated on GD 6-10 at 75 mg/kg/day had a 75% incidence of FLR, but rats treated on GD 11-15 at 75 or 100 mg/kg/day were unaffected. Third, 24 h after a single dose, all dams with FLR had markedly reduced serum progesterone levels; however, LH levels were unaffected. The high FLR rate during the LH-dependent period, the lack of response thereafter, and the reduced progesterone levels without an associated reduction in LH levels suggests that BDCM disrupts luteal responsiveness to LH.

Effect of dosing vehicle on the developmental toxicity of bromodichloromethane and carbon tetrachloride in rats.

Narotsky M. G., Pegram R. A. and Kavlock R. J.
Fundamental and Applied Toxicology 1997 40(1):30-6.

Several halocarbons have been shown to cause full-litter resorption (FLR) in Fischer-344 rats when administered orally in corn oil. Since halocarbons often occur as contaminants of drinking water, we sought to determine the influence of the vehicle, aqueous versus lipid, on the developmental toxicity of two of these agents. In separate assays, bromodichloromethane (BDCM) and carbon tetrachloride (CCl₄) were administered by gavage to Fischer-344 rats on gestation days (GD) 6-15 at 0, 25, 50, or 75 mg/kg/day in either corn oil or an aqueous vehicle containing 10% Emulphor EL-620. Dams were allowed to deliver and the litters were examined

postnatally. Uteri of females that did not deliver were stained with 10% ammonium sulfide to detect FLR. Effects of both agents on maternal weight gain were slightly more pronounced in the aqueous vehicle at lower doses, but at the highest dose, CCl₄ was more maternally toxic in corn oil. Developmentally, both agents caused FLR at 50 and 75 mg/kg in both vehicles. At 75 mg/kg, dams receiving corn oil had significantly higher rates of FLR (83% for BDCM, 67% for CCl₄) compared to their aqueous-vehicle counterparts (21% for BDCM, 8% for CCl₄). Blood concentrations of BDCM following GD-6 gavage revealed a shorter elimination half-life in the aqueous dosing vehicle (2.7 h) compared to the oil vehicle (3.6 h). Benchmark doses of CCl₄ were similar for the oil (18.9 mg/kg) and aqueous (14.0 mg/kg) vehicles. For BDCM, the corn oil vehicle yielded a less conservative (i.e., higher) value (39.3 mg/kg) than the aqueous vehicle (11.3 mg/kg), reflecting different confidence intervals around the estimated 5%-effect dose levels.

Preliminary screening for the potential of drinking water disinfection byproducts to alter male reproduction.

Klinefelter G. R., Suarez J. D., Roberts N. L. and DeAngelo A. B.
Reproductive Toxicology 1995 9(6):571-8.

There is increasing epidemiologic interest in the role drinking water disinfection byproducts (DBPs) may play in adverse reproductive outcomes such as inability to conceive, spontaneous abortion, and low birth weight. Although dozens of DBPs already have been identified, only a few studies have attempted to determine whether DBPs alter male reproductive parameters such as testicular and epididymal histology, testicular and epididymal sperm numbers, and epididymal sperm morphology and motility in laboratory animals. In these studies, alterations in epididymal sperm motility seemed to be predictive of more generalized toxicity of the male reproductive system. Because there is a need to prioritize DBPs for thorough reproductive and developmental toxicity testing, preliminary screening for the potential of DBPs to alter reproductive function seems warranted. Here, we elected to examine only cauda epididymal sperm motion parameters and testicular and epididymal histopathology. The effects of exposure to two commonly occurring DBPs, bromodichloromethane (BDCM) and chloral hydrate (CH), via drinking water were evaluated in F344 rats at an interim (52 week) necropsy during cancer bioassay studies. Exposure to 22 and 39 mg/kg BDCM and 55 and 188 mg/kg CH did not produce any systemic toxicity. Histopathologic evaluation revealed no gross lesions in the reproductive organs, and no tumors were detected in any tissues. In contrast, exposure to 39 mg/kg BDCM significantly decreased the mean straight-line, average path, and curvilinear velocities of sperm recovered from the cauda epididymidis. This BDCM exposure shifted the average path velocity distribution to a lower modal velocity range. Exposure to 188 mg/kg CH significantly decreased both the percentage of motile and progressively motile sperm. This CH exposure shifted the straight-line velocity distribution to a lower modal velocity range. These are the first reproductive toxicity data from exposure to BDCM and CH. The observed effects on sperm motion occurred in the absence of carcinogenesis. Because the effects of BDCM on sperm motility occurred at a lower exposure than that of other DBPs that compromise sperm motility, a thorough reproductive evaluation now is underway.

A.3 DBCM

No abstracts were identified in this category.

A.4 Bromoform

Reproductive toxicology. Bromoform.

Chapin R. G., D., Hope E., Mounce R., Russell S. and Poonacha K. B.
Environmental Health Perspectives 1997 105 Suppl 1:277-8.

Bromoform, which is present in drinking water supplies at low microgram to nanogram levels, was tested for its effects on reproduction and fertility in Swiss CD-1 mice, following the RACB protocol. Data on body weights, clinical signs, and food and water consumption from a 2-week dose-range-finding study (Task 1) were used to set exposure levels for the Task 2 continuous cohabitation phase at 50, 100, and 200 mg/kg/day by gavage. With a single exception, dosing formulations were 90 to 110% of nominal concentrations.

During Task 2 six animals died from parturition complications, infection, and skin wounds inflicted by the partner, which occurred in all dose groups and were not related to treatment. Water consumption was increased for high dose animals by approximately 16%. Starting with the first litter, postpartum dam weights were lower in the high dose group, compared to controls, by 3 to 8%. Other dose levels, and male body weights, were unaffected.

In Task 2, there were no treatment-related changes in reproductive end points: number of litters per pair, live pups per litter, viability, and pup body weights were all unaffected by these levels of bromoform exposure. The average study day for litter delivery was not affected by treatment. In the absence of a reproductive effect to investigate, no crossover tests were performed. The last litters from all dose groups were nursed by their dams until weaning. Bromoform exposure increased neonatal mortality by 15 to 20% at the high dose only in the first 4 days postnatally. Pup body weight was occasionally significantly reduced in high dose pups prior to weaning.

At weaning, the F₀ mice were killed and discarded without necropsy. Following the protocol of a "negative" study, at weaning the pups from the low and middle dose groups were killed and discarded, and the pups from the control and high dose groups were reared and dosed through the mating period (at 74 ± 10 days of age) until necropsy.

During the F₁ mating trial, there were no differences between the two groups with respect to mating or fertility index. The number, viability, and weight of pups were not affected by bromoform exposure.

After the F₂ pups were delivered and evaluated, the F₁ adults were killed and necropsied. While female body weights were unaffected by exposure to 200 mg/kg/day bromoform, relative liver weight was increased by 8%, and relative kidney weight was decreased by 6%. Vaginal cyclicity was not examined in these mice. In males, body weights of the exposed mice were reduced by 6%, while weight-adjusted liver weights were increased by 6% and adjusted kidney weights were reduced by 6%. There were no changes in other organ weights or in epididymal sperm parameters.

Microscopically, varying degrees of hepatocellular degeneration was seen in all treated male and female mice. No treatment-related alterations were noted in kidney, thyroid, lung, or sex organs. In summary, bromoform caused a slight increase in postnatal mortality, but no changes in other reproductive indices, at doses that caused significant hepatotoxicity.

A teratological assessment of four trihalomethanes in the rat.

Ruddick J. A., Villeneuve D. C., Chu I. and Valli V. E.

Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes 1983 18(3):333-49.

Four trihalomethanes were administered by gavage to Sprague-Dawley rats from day 6 to day 15 of gestation. Chloroform (Ch) was administered at levels of 100, 200 and 400 mg/kg and bromoform (Br), bromodichloromethane (BDCM) and chlorodibromomethane (CDBM) were administered at levels of 50, 100 or 200 mg/kg/day. A separate control was used for each compound. Maternal weight gain was depressed in all groups receiving Ch and at the highest dose levels of BDCM and CDBM. Ch administration caused decreased maternal hemoglobin and hematocrit values at all dose levels and also produced increased serum inorganic phosphorus and cholesterol at the highest dose. Liver enlargement was observed at all dose levels of Ch but in no other treatment groups. Evidence of a fetotoxic response was observed with Ch, CDBM and Br but not BDCM. No dose-related histopathological changes were observed in either mothers or fetuses as a result of treatment. None of the chemicals tested produced any teratogenic effects.

A.5 Chloroform

The effects of in utero and lactational exposure to chloroform on postnatal growth and glucose tolerance in male Wistar rats.

Lim G. E., Stals S. I., Petrik J. J., Foster W. G. and Holloway A. C.

Endocrine. 2004 25(3):223-8.

Water chlorination results in the formation of trihalomethanes (THMs) including chloroform. In human studies, fetal growth restriction has been associated with exposure to THMs during pregnancy and impaired fetal growth has been associated with an increased risk of type 2 diabetes. Therefore, the objective of this study was to determine the effect of in utero and lactational exposure to chloroform on birthweight and postnatal indicators of type 2 diabetes. Female Wistar rats were given chloroform (0 microg/L, 75 microg/L) in their drinking water for 2 wk prior to mating until parturition (in utero exposure only) or until weaning (in utero+lactational exposure). At postnatal d 1 (PND1) pups of dams exposed to chloroform had significantly higher serum glucose levels and lower insulin levels, but this effect was not due to β -cell depletion in the neonatal pancreas. Glucose homeostasis in response to a glucose challenge was not changed by chloroform treatment. Chloroform exposure did not affect birthweight; however, offspring of dams exposed to chloroform had significantly impaired postnatal growth. Although fetal and neonatal exposure to chloroform did not elicit physiological changes associated with the onset of type 2 diabetes, there were physiological changes resulting in impaired postnatal growth.

In vitro assessment of the effect of halogenated hydrocarbons: chloroform, dichloromethane, and dibromoethane on embryonic development of the rat.

Brown-Woodman P. D., Hayes L. C., Huq F., Herlihy C., Picker K. and Webster W. S. *Teratology* 1998 57(6):321-33.

Halogenated hydrocarbons are widely used in industry, the laboratory, and in the home. In the present study three of these solvents--chloroform, dichloromethane, and dibromoethane--were examined for embryotoxic/teratogenic potential using rat embryo culture. The results showed that each of the solvents had a concentration-dependent embryotoxic effect on the developing rat embryo in vitro. The effect and no-effect concentrations (expressed in $\mu\text{mol/ml}$ culture medium), respectively, for each of the halogenated hydrocarbons tested were: dibromoethane--0.33, of chloroform and dichloromethane found to be embryotoxic in the present study were compared to reported blood levels attained following controlled human exposure. In the industrial situation, if the current exposure levels are adhered to, chloroform and dichloromethane appear to have little potential for reproductive toxicity in the human. Fatal or near fatal solvent levels would be required in the mother for the embryotoxic level to be reached. For dibromoethane, there are no reports following controlled human exposure presumably due to its carcinogenicity. In an attempt to elucidate the mechanism of embryotoxicity, histological studies were performed after exposure of rat embryos to an embryotoxic level of each of the halogenated hydrocarbons studied, for increasing time periods up to the standard 40-hour culture. Marked cell death in the neuroepithelium of the developing neural tube was a prominent feature in all embryos exposed to an embryotoxic level of these solvents for periods of 16 hours or longer.

A teratological assessment of four trihalomethanes in the rat.

Ruddick J. A., Villeneuve D. C., Chu I. and Valli V. E. *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes* 1983;18(3):333-49.

Four trihalomethanes were administered by gavage to Sprague-Dawley rats from day 6 to day 15 of gestation. Chloroform (Ch) was administered at levels of 100, 200 and 400 mg/kg and bromoform (Br), bromodichloromethane (BDCM) and chlorodibromomethane (CDBM) were administered at levels of 50, 100 or 200 mg/kg/day. A separate control was used for each compound. Maternal weight gain was depressed in all groups receiving Ch and at the highest dose levels of BDCM and CDBM. Ch administration caused decreased maternal hemoglobin and hematocrit values at all dose levels and also produced increased serum inorganic phosphorus and cholesterol at the highest dose. Liver enlargement was observed at all dose levels of Ch but in no other treatment groups. Evidence of a fetotoxic response was observed with Ch, CDBM and Br but not BDCM. No dose-related histopathological changes were observed in either mothers or fetuses as a result of treatment. None of the chemicals tested produced any teratogenic effects.

B. Studies reporting no developmental or reproductive toxicity

B.1 Total Trihalomethanes

Behavioral toxicity of trihalomethane contaminants of drinking water in mice.

Balster R. L. and Borzelleca J. F.

Environmental Health Perspectives 1982 46:127-36.

The behavioral toxicity of trichloromethane (TCM), dichlorobromomethane (DCBM), dibromochloromethane (DBCM) and tribromomethane (TBM) was evaluated following oral administration in mice. A variety of dosage regimens and behavioral measures were used. Studies included acute dose effect, 14- and 90-day treatments at 300 and 3000 times the estimated average human daily intake of contaminated drinking water, 30 days of 100 mg/kg/day, and 60 days of 100 and 400 mg/kg/day. In addition, TCM was tested for the production of taste aversions with 10-day administration and for behavioral teratology in offspring following extensive perinatal exposure. The ED₅₀ for acute effects on a screen test of motor performance was about 500 mg/kg for all four trihalomethanes. The 14-day treatments had no effect on swimming behavior and the 90-day treatments had no effect on bar clinging, a test of motor coordination, and a measure of exploratory behavior. None of the compounds produced effects on passive-avoidance learning following 100 mg/kg/day for 30 days. TCM, DBCM and TBM elicited clear effects at both 100 and 400 mg/kg/day on operant behavior when administered for 60 days. DBCM elicited clear effects at 400 mg/kg/day. These effects on operant behavior were seen following the first dose and tolerance tended to develop. Thus, there was no evidence from these studies for a progressive neurotoxicity from trihalomethanes in adult mice. A behavioral teratology study was also conducted with TCM. Both parents were treated with 31.1 mg/kg/day TCM, and treatment of the dam continued throughout gestation and lactation. No clear evidence for behavioral effects in the offspring were observed. The most sensitive measure for the effects of TCM was the taste aversion paradigm in which saccharin aversions were produced after a single treatment of 30 mg/kg.

B.2 BDCM

Oral (drinking water) two-generation reproductive toxicity study of bromodichloromethane (BDCM) in rats.

Christian M. S., York R. G., Hoberman A. M., Fisher L. C. and Brown W. R.

International Journal of Toxicology 2002 21(2):115-46.

Bromodichloromethane (BDCM) was tested for reproductive toxicity in a two-generation study in CRL SD rats. Thirty rats/sex/ group/generation were continuously provided BDCM in drinking water at 0 (control carrier, reverse osmosis membrane-processed water), 50, 150, and 450 ppm (0, 4.1 to 12.6, 11.6 to 40.2, and 29.5 to 109.0 mg/kg/day, respectively). Adult human intake approximates 0.8 microg/kg/day (0.0008 mg/kg/day). P and F1 rats were observed for general toxicity (viability, clinical signs, water and feed consumption, body weights, organ

weights [also three weanling F1 and F2 pups/sex/litter], histopathology [10/sex, 0- and 450-ppm exposure groups]) and reproduction (mating, fertility, abortions, premature deliveries, durations of gestation, litter sizes, sex ratios, viabilities, maternal behaviors, reproductive organ weights [also three weanling F1 and F2 pups/sex/ litter], sperm parameters, and implantations. F1 rats were evaluated for age at vaginal patency or preputial separation. Ten P and F1 rats/sex from the 0- and 450-ppm exposure groups and rats at 50 and 150 ppm with reduced fertility were evaluated for histopathology (gross lesions, testes, intact epididymis, all F1 dams for number of primordial follicles). Developmental parameters in offspring included implantation and pup numbers, sexes, viabilities, body weights, gross external alterations, and reproductive parameters (F1 adults). Toxicologically important, statistically significant effects at 150 and/or 450 ppm included mortality and clinical signs associated with reduced absolute and relative water consumption, reduced body weights and weight gains, and reduced absolute and relative feed consumption (P and F1 rats). Significantly reduced body weights at 150 and 450 ppm were associated with reduced organ weights and increased organ weight ratios (% body and/or brain weight). Histopathology did not identify abnormalities. Small delays in sexual maturation (preputial separation, vaginal patency) and more F1 rats with prolonged diestrus were also attributable to severely reduced pup body weights. Mating, fertility, sperm parameters, and primordial ovarian follicular counts were unaffected. The no-observable-adverse-effect level (NOAEL) and the reproductive and developmental NOAELs for BDCM were at least 50 ppm (4.1 to 12.6 mg/kg/day), 5125 to 15,750 times the human adult exposure level, if delayed sexual maturation associated with severely reduced body weights is considered reproductive toxicity. If considered general toxicity, reproductive and developmental NOAELs for BDCM are greater than 450 ppm (29.5 to 109.0 mg/kg/day), or 36,875 to 136,250 times the human adult exposure level. Regardless, these data indicate that BDCM should not be identified as a risk to human reproductive performance or development of human conceptuses.

Oral (drinking water) developmental toxicity studies of bromodichloromethane (BDCM) in rats and rabbits.

Christian M. S., York R. G., Hoberman A. M., Diener R. M. and Fisher L. C.
International Journal of Toxicology 2001 20(4):225-37.

Crl:CD(SD)IGS BR VAF/Plus (Crl SD) rats and Hra(NZW) SPF rabbits were tested for potential developmental toxicity from bromodichloromethane (BDCM) provided continuously in the drinking water during gestation (gestation days [GDs] 6 to 21 in rats and GDs 6 to 29 in rabbits). Concentrations of 0, 50, 150, 450, or 900 ppm of BDCM were used for rats; 0, 15, 150, 450, or 900 ppm were used for rabbits (in dose range-finding studies, 1350 ppm was excessively maternotoxic to both species). Investigated maternal parameters included viability, clinical signs, water and feed consumption, and body weights. Maternal gross lesions, gravid uterine weights, abnormal placentas, and numbers of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions were observed at time of Caesarean sectioning (GD 21 in rats; GD 29 in rabbits). Body weights, sex ratios, and morphological abnormalities (external, soft tissue, and skeletal) were noted in the fetuses. Mean consumed doses of BDCM were calculated to be 0, 2.2, 18.4, 45.0, or 82.0 mg/kg/day for the rats, and 0, 1.4, 13.4, 35.6, or 55.3 mg/kg/day for the rabbits (approximate human intake is 0.8 microg/kg/day [0.0008 mg/kg/day] in adults). In

pregnant rats, toxicologically important, statistically significant effects included reduced absolute (g/day) and relative (g/kg/day) water consumption values at > or =50 ppm (2.2 mg/kg/day) and reduced body weight gains (also when corrected for gravid uterine weight) and absolute (g/day) and relative (g/kg/day) feed consumption values at >450 ppm (45.0 mg/kg/day). These parameters were also significantly reduced at > or =450 ppm (35.6 mg/kg/day) in pregnant rabbits (significant weight loss occurred in the rabbits at 900 ppm, i.e., 55.3 mg/kg/day). Thus, the maternal no-observable-adverse-effect level (NOAEL) for BDCM was 150 ppm, i.e., 18.4 and 13.4 mg/kg/day in rats and rabbits, respectively. No adverse effects on embryofetal viability, growth, sex ratio, gross external, soft tissue, or skeletal morphology occurred at 900 ppm in rats or rabbits. Minimal delays in the ossification of forepaw phalanges and hindpaw metatarsals and phalanges occurred in rat fetuses at 900 ppm; delays were considered marginal, reversible, and associated with severely reduced maternal weight gain. Therefore, the developmental NOAEL for rats was 450 ppm (45.0 mg/kg/day), whereas in rabbits it was 900 ppm (55.3 mg/kg/day). These NOAELs are 56,250 and 69,120 times the human adult exposure level of 0.0008 mg/kg/day, respectively. Based on the results of these studies, BDCM should not be identified as a risk to development of human conceptuses.

Biodisposition of dibromoacetic acid (DBA) and bromodichloromethane (BDCM) administered to rats and rabbits in drinking water during range-finding reproduction and developmental toxicity studies.

Christian M. S., York R. G., Hoberman A. M., Diener R. M., Fisher L. C. and Gates G. A. International Journal of Toxicology 2001 20(4):239-53.

Dibromoacetic acid (DBA) and bromodichloromethane (BDCM), by-products of chlorine disinfection of water, were provided in drinking water in range-finding reproductive/developmental toxicity studies (rats) and a developmental toxicity study (BDCM) in rabbits. Studies included absorption and biodisposition of DBA and BDCM, including passage into placentas, amniotic fluid, fetuses (rats and rabbits), or milk (rats). The DBA and BDCM range-finding reproductive/developmental toxicity studies each included 50 Sprague-Dawley rats/sex/group. DBA (0, 125, 250, 500, or 1000 ppm) or BDCM (0, 50, 150, 450, or 1350 ppm) was provided in drinking water 14 days pre-mating through gestation and lactation (63 to 70 days). The developmental toxicity range-finding study included 25 time-mated New Zealand white rabbits/group given 0, 50, 150, 450, or 1350 ppm BDCM in drinking water on gestation days (GDs) 6 through 29. Satellite groups (6 male, 17 female rats/group/study and 4 rabbits/group) were used for bioanalytical sampling. Rats and rabbits had exposure-related reduced water consumption caused by apparent taste aversion to DBA or BDCM, especially in the parental animals at the two highest exposure levels (500 and 1000 ppm DBA; 450 and 1350 ppm BDCM). Female rats consumed slightly higher mg/kg/day doses of DBA than male rats, especially during gestation and lactation; weanling rats consumed the highest mg/kg/day doses. DBA produced detectable and quantifiable concentrations in plasma, placentas, amniotic fluid, and milk. Plasma samples confirmed that rats drink predominately during the dark; this drinking pattern, not accumulation, produced detectable plasma concentrations for 18 to 24 hours/day. No quantifiable concentrations of BDCM occurred in plasma, placentas, amniotic fluid, or milk, suggesting that BDCM is rapidly degraded or metabolized in vivo. DBA (500 and 1000 ppm,

rats) and BDCM (450 and 1350 ppm, rats and rabbits) produced secondary toxicity in the parental generation by reducing water consumption, which caused severe exposure-related apparent dehydration, reduced feed intake and weight gain. Reproductive and developmental parameters were essentially unaffected (mating possibly reduced [DBA at 1000 ppm]; exposure-related decreases in body weights of pups secondary to reduced water and feed consumption [DBA at 250, 500, and 1000 ppm; BDCM at 150, 450, and 1350 ppm]). No effects on development of rabbit fetuses occurred at BDCM concentrations as high as 1350 ppm. Results from these preliminary studies, in which DBA and BDCM were provided in the drinking water at concentrations thousands of times higher than those to which humans are exposed, suggest that neither DBA nor BDCM are reproductive/developmental risks for humans.

Final report on the short term reproductive and developmental toxicity of bromodichloromethane (CAS #75-27-4) administered in drinking water to Sprague-Dawley rats.

Wolfe G. W. and et al.

NTIS Technical Report 1998 111262(94017).

The potential toxicity of bromodichloromethane (BDCM; CAS No. 75-27-4) was evaluated using a short-term reproductive and developmental toxicity screen. This study design was selected to identify the physiologic process (development; female reproduction; male reproduction; various somatic organs/processes) that is the most sensitive to bromodichloromethane exposure. The dose range-finding study was conducted at concentrations of 0, 100, 500, 1000, and 1500 ppm of bromodichloromethane in the drinking water for two weeks. Based on decreases in water consumption, concentrations of 0, 100, 700, and 1300 ppm (Groups 1, 2, 3, and 4, respectively) were selected for the main study. The main study utilized two groups of male rats designated as Group A (non-BrdU treated animals, 10 per group in Groups 1-4) and Group B (BrdU treated, 5 animals in Groups 1, 2, and 3, and 8 animals in Group 4), and three groups of female rats designated as Group A (peri-conception exposure, 10 per group in Groups 1-4), Group B (gestational exposure, 13 per group in Groups 1-4), and Group C (peri-conception exposure, BrdU-treated, 5 animals in Groups 1, 2, and 3, and 8 animals in Group 4). Control animals received deionized water, the vehicle. During the treatment period, all animals except one survived to the scheduled necropsy. Body weights and feed and water consumption were decreased at many of the intervals for the 700 and 1300 ppm dose groups. Body weights were decreased by 5-13% compared to the controls at many of the intervals while feed consumption was decreased 14-47%, and water consumption was decreased by 17-86% at many of the intervals. The overall calculated mean consumption of BDCM for Groups 2-4 was 11, 53, and 88 mg/kg/day, respectively. At necropsy, clinical chemistry and hematology endpoints were unaffected by BDCM treatment except for a 43% increase in the 5'-Nucleotidase in the 1300 ppm A males, which most likely represents interference with the secretion of bile, and a 14% decrease in creatinine in the 100 ppm A males. Necropsy organ weights and organ-to-body weight ratios were comparable to the controls. Gross findings were comparable across all groups. Microscopically, cytoplasmic vacuolization of hepatocytes and individual hepatocyte necrosis were observed in tissues from the 700 and 1300 ppm A males and the 1300 ppm B males, indicative of mild liver damage. Hematopoietic cell proliferation in the spleen was

observed in tissues from all dosed A males, but this is most likely an indirect change in response to stress (See Discussion). The Labeling Index (LI) for the liver and kidney from the B males were relatively comparable between treated and control groups, but the LI for the liver and kidneys from the 1300 ppm C females were significantly increased, indicating possible early stimulation of cellular proliferation. There were no treatment-related findings noted in any male and female reproductive parameters. Results of this study indicate that BDCM at doses at and above 700 ppm produced consistent decreases in body weight and food and water consumption in both sexes, but did not result in any male or female reproductive toxicity. From these data, BDCM is taste-aversive and a general toxicant in both sexes at doses at and above 700 ppm.

A teratological assessment of four trihalomethanes in the rat.

Ruddick J. A., Villeneuve D. C., Chu I. and Valli V. E.

Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes 1983 18(3):333-49.

Four trihalomethanes were administered by gavage to Sprague-Dawley rats from day 6 to day 15 of gestation. Chloroform (Ch) was administered at levels of 100, 200 and 400 mg/kg and bromoform (Br), bromodichloromethane (BDCM) and chlorodibromomethane (CDBM) were administered at levels of 50, 100 or 200 mg/kg/day. A separate control was used for each compound. Maternal weight gain was depressed in all groups receiving Ch and at the highest dose levels of BDCM and CDBM. Ch administration caused decreased maternal hemoglobin and hematocrit values at all dose levels and also produced increased serum inorganic phosphorus and cholesterol at the highest dose. Liver enlargement was observed at all dose levels of Ch but in no other treatment groups. Evidence of a fetotoxic response was observed with Ch, CDBM and Br but not BDCM. No dose-related histopathological changes were observed in either mothers or fetuses as a result of treatment. None of the chemicals tested produced any teratogenic effects.

B.3 DBCM

No abstracts were identified in this category.

B.4 Bromoform

No abstracts were identified in this category.

B.5 Chloroform

No abstracts were identified in this category.

C. Related articles and meeting abstracts

C.1 Total Trihalomethanes

Effects of Defined Mixtures of Trihalomethanes and Haloacetic Acids on Pregnancy Maintenance and Eye Development in F344 Rats.

Narotsky M. G., Best D. S., McDonald A., Myers E. A., Hunter E. S. d. and Simmons J. E. Birth Defects Research. Part A, Clinical and Molecular Teratology 2006 76(5):384.

Although disinfection of drinking water is important for control of microbial contamination, it results in the formation of hundreds of disinfection by-products (DBPs). The most prevalent DBPs are trihalomethanes (THMs; chloroform, bromodichloromethane, chlorodibromomethane, bromoform) and haloacetic acids (HAAs; chloroacetic, dichloroacetic, trichloroacetic, bromoacetic, and dibromoacetic acid). THMs and HAAs are regulated in drinking water at 80 and 60 ug/L, respectively. In rats, THMs have been shown to cause pregnancy loss (i.e., full-litter resorption, an all-or-none effect). HAAs have been shown to cause eye defects and partial-litter, as well as full-litter, resorption (i.e., not an all-or-none effect). Here, we assessed the combined toxicity of these DBPs. Rats were treated with mixtures of four THMs (THM4), five BAAs (HAA5), or nine DBPs (DBP9; THM4 + HAA5). Chemical proportions reflected those in tap water; e.g., in DBP9, molar percentages of the respective chemicals were 30.0, 11.9, 7.0, 1.0, 1.7, 22.7, 15.9, 8.2, and 1.5. Mixtures, prepared in 10% Alkamuls®; EL-620, were administered daily to F344 rats by gavage on gestation days 6-20. Litters were examined on postnatal days 1 and 6. For the THM4 mixture, pregnancy loss was seen in 0/14, 0/25, 11/14 (79%), and 12/13 (92%) of the dams at 0, 307, 613, and 920 umol/kg, respectively. Pup weights were reduced at 613 and 920 umol/kg. Postnatal loss was increased at 920 umol/kg. For the HAA5 mixture, pregnancy loss was seen in 0/9, 0/15, 3/17 (18%), and 11/11 (100%) of the dams at 0, 308, 615, and 1231 umol/kg, respectively. Eye malformations (anophthalmia or microphthalmia) were seen in 0, 53%, and 79% of the live litters at 0, 308, and 615 umol/kg. Prenatal loss was unaffected in live litters. For the DBP9 mixture, pregnancy loss was seen in 0/18, 0/19, 6/17 (35%), and 7/8 (88%) of the dams at 0, 307, 615, and 1228 umol/kg, respectively. In live litters, prenatal loss was unaffected. Thus, THM4, HAA5, and DBP9 each caused pregnancy loss at ≥ 613 umol/kg; i.e., both HAAs and THMs contributed to DBP9-induced pregnancy loss. The presence of THMs in the full mixture, however, reduced the incidence of BAA-induced eye defects.

Effects of Defined Mixtures of Trihalomethanes and Haloacetic Acids on Pregnancy Maintenance in F344 Rats.

Narotsky M. G., Best D. S., McDonald A., Myers E. A., Hunter E. S. and Simmons J. E. Birth Defects Research. Part A, Clinical and Molecular Teratology 2005 73(5):358.

Although disinfection of drinking water is vitally important for eliminating microbial contamination, it also causes formation of hundreds of disinfection by-products (DBPs). The most prevalent DBPs are trihalomethanes (THMs; chloroform, bromodichloromethane, chlorodibromomethane, bromoform) and haloacetic acids (HAAs; chloroacetic, dichloroacetic,

trichloroacetic, bromoacetic, and dibromoacetic acids). Some epidemiology studies have shown an increased risk of spontaneous abortion associated with consumption of water with high concentrations of THMs. We have previously shown that bromodichloromethane causes pregnancy loss, i.e., full-litter resorption, in F344 rats via a luteinizing hormone-mediated mechanism (low-effect level = 305 umol/kg). HAAs, however, have not been quantified in these epidemiology studies, nor have they been tested in the F344 strain for their effects during gestation. In this study, we tested mixtures of the four THMs (THM4) as well as mixtures of the THMs and HAAs (DBP9; THM4 HAA5). Chemical proportions mimicked those found in drinking water. The DBP9 mixture consisted of ~50% THM4 and ~50% HAA5. Mixtures, prepared in 10% Alkamuls® EL- 620, were administered to F344 rats by gavage on gestation days 6-20. Litters were examined on postnatal days 1 and 6. For the THM4 mixtures, pregnancy loss was seen in 0/5, 0/10, 11/14 (79%), and 12/13 (92%) of the dams at 0, 307, 613, and 920 umol/kg, respectively. Among surviving litters, pup weights were reduced at 613 and 920 umol/kg. Postnatal loss was increased at 920 umol/kg. For the DBP9 mixtures, pregnancy loss was seen in 0/18, 0/19, 6/17 (35%), and 7/8 (88%) of the dams at 0, 307, 615, and 1228 umol/kg, respectively; no effects on pup weight or postnatal mortality were observed. It is noteworthy that all dams maintained pregnancy at 307 umol THM4/kg; however, the addition of HAA5 to this mixture (i.e., 307 umol THM4/kg 308 umol HAA5/kg 615 umol DBP9/ kg) resulted in pregnancy loss. This finding suggests that either HAAs cause pregnancy loss in F344 rats, or that HAAs potentiate the THMs’ disruptive effect on pregnancy. We plan to evaluate HAAs for their effects on pregnancy in the F344 rat.

Fetal Exposure To Water Disinfection By-Products Alters Postnatal Growth And Glucose Homeostasis.

Lim G. E., Stals S. I., Foster W. G., Petrik J. J. and Holloway A. C.

Journal of the Society for Gynecologic Investigation 2004 11(2 Suppl).

Introduction: Epidemiological studies reveal that in utero insults resulting in impaired fetal growth have long-term consequences for postnatal health including an increased risk of type 2 diabetes as adults. It has been well documented that chlorination of drinking water results in the formation of trihalomethanes (THM). Epidemiological studies have shown that exposure to THMs during pregnancy may result in adverse developmental effects including fetal growth restriction. Given the importance of birthweight as a predictor of adult health in human populations, and the association between in utero exposure to THMs and intrauterine growth restriction, we therefore propose that fetal exposure to THMs may have life-long health consequences, which have not been previously identified. Objective: In this study, we examined the effect of in utero exposure to chloroform, the most predominate THM, on fetal and postnatal growth, and postnatal glucose homeostasis. Methods: Female nulliparous Wistar rats were given chloroform in their drinking water during pregnancy through to weaning at concentrations of 0ug/L, 75ug/L, and 150ug/L, which are representative of levels found in North American public water supplies. Dams were allowed to deliver normally, and the litters were culled to three males which remained with the dam until weaning. Serum and pancreas tissue were collected from the remaining animals. At 4 weeks of age, pups were challenged with an oral glucose load to evaluate serum glucose, and fasting serum glucose was measured at 15 weeks of age. Results:

Birth weight in animals exposed to chloroform in utero was reduced relative to control animals but this effect did not reach significance. At weaning, body weights of both chloroform treatment groups were significantly lower than control animals. This effect of chloroform to inhibit postnatal growth persisted in the 75ug/l group until 22 weeks of age, and until 8 weeks of age body in the 150ug/l group. At PND1, serum glucose concentrations were elevated in pups born to chloroform exposed dams. This effect had disappeared by 4 weeks of age, and there was no difference between groups in the response to an oral glucose load at 4 weeks of age. At 15 weeks of age however, fasting serum glucose concentrations were significantly lower in pups from the 75ug/L treatment group. Conclusion: These data show for the first time that fetal and neonatal exposure to chloroform at concentrations found in North American public drinking water supplies can inhibit postnatal growth and alter glucose homeostasis in juvenile animals. We propose that THM exposure in utero and postnatally may have a profound effect on children's health which has not been fully explored.

Animal models for studying miscarriage: illustration with study of drinking water disinfection byproducts.

Narotsky M. G. and Laffan S. B.

Birth Defects Res Part A Clin Mol Teratol 2004 70(5):283.

No single animal model is ideal for investigating spontaneous abortion. However, different species (e.g., rat, mouse, monkey) do offer research opportunities that can provide insights into possible causes of miscarriage. In the F344 rat, several chemicals [e.g., trichloroethylene, atrazine, bromodichloromethane (BDCM)] have been shown to cause pregnancy loss, i.e., full-litter resorption (FLR). BDCM, a drinking water disinfection byproduct, is of particular interest because of epidemiological evidence associating it with spontaneous abortion. In the rat, BDCM's disruption of pregnancy is an all-or-none effect in that surviving litters appear to be unaffected. The F344 strain is particularly sensitive to this effect whereas the Long-Evans rat is moderately sensitive and the Sprague Dawley rat appears to be insensitive. Several lines of evidence indicate that BDCM disrupts pregnancy in the rat by a luteinizing hormone (LH)-mediated mode of action: 1) BDCM's effect requires exposure during the LH-dependent period of pregnancy. 2) BDCM decreases serum levels of LH (and progesterone) in rats with FLR. 3) Treatment with hCG, an LH agonist, rescues BDCM-exposed pregnancies from FLR. 4) Corpora lutea cultured in BDCM are less responsive to hCG stimulation in vitro. Given that LH and hCG bind to the same luteal receptor, responsiveness to LH in rats is analogous to responsiveness to hCG in humans. Regarding LH secretion, if BDCM disrupts LH secretion in the rat via altered GnRH signaling, this could be relevant to human pregnancy in that placental secretion of hCG is also regulated by GnRH. Indeed, recent data demonstrate that BDCM inhibits human placental trophoblast differentiation and hCG secretion in vitro. Thus, animal models may give important insights into the causes of miscarriage, and offer guidance for further research.

Effects of bromodichloromethane (BDCM) on ex vivo luteal function in the pregnant F344 rat.

Bielmeier S. R., Murr A. S., Best D. S., Goldman J. M. and Narotsky M. G.
Toxicologist 2003 72(S-1):26-7.

We have reported that BDCM, a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption, in F344 rats when treated on gestation day (GD) 6-10, encompassing the luteinizing hormone (LH)-dependent period. BDCM-induced pregnancy loss was associated with reductions in serum progesterone (P) and corresponding decreases in LH on GD 10, suggesting BDCM disrupts the maternal hypothalamic-pituitary-gonadal axis. These and other data indicate that BDCM affects the hypothalamus or pituitary gland; however, an effect on luteal responsiveness to LH had not been definitively excluded. To address this data gap, we used an ex vivo approach to assess luteal function. Dams were dosed by gavage on GD 6-9 (plug day = GD 0) at 0 or 100 mg/kg/d (n = 11, 12). One hour after the GD-9 dose, rats were sacrificed, blood was collected and corpora lutea (CL) were incubated with or without hCG, an LH agonist, to stimulate P secretion. During the 24 h incubation, media were periodically sampled for hormone analysis by dissociation enhanced lanthanide fluorescence immunoassay (DELFIATM). Luteal responsiveness was unaffected; both groups displayed a 2.4-fold increase in P secretion in response to hCG challenge. Paradoxically, the BDCM-exposed CL showed greater than 2-fold increases in P secretion ex vivo regardless of the presence of hCG; whereas the same animals, i.e., the CL donors, had decreased serum P and LH levels in vivo. It is unclear if this 'rebound' effect reflects the removal of the CL from a possible direct inhibitory influence of BDCM, or a response to the diminished LH stimulation in vivo. Regardless, the lack of effect on luteal responsiveness is further evidence that BDCM-induced pregnancy loss in the rat is due to reduced pituitary LH secretion.

Effects on the gonadal development of medaka (*Oryzias latipes*) exposed as embryos to bromodichloromethane.

Thiyagarajah A., Parker S., Gennings C., Teuschler L. E., Conerly O. and Hartley W. R.
Toxicologist 2002 66(1-S):374-5.

The purpose of this study is to determine the toxic effects of bromodichloromethane (BDCM) on the developing gonad of medaka. BDCM is one of the trihalomethanes, a disinfection by-product found in the drinking water with developmental/reproductive toxicity concerns expressed in epidemiological studies. Medaka embryos at early high blastula stage were exposed to 10 or 25 mg/L BDCM for 10 days, and transferred to contaminant-free embryo rearing medium until hatched. Upon hatching, medaka fry were grown-out in spring water for 6 and 12 months. Two controls (embryo-rearing solution and DMSO carrier) were used. Fish were sampled at 6 and 12-month post-exposure and processed for histological evaluation. The evaluated endpoints, indicative of endocrine disruption or other reproductive toxicities were: hermaphroditism; immature gonads; ectopic gonads; male:female ratio; and other pathology. Hermaphroditism was not observed. There were two cases of ectopic gonads (10 mg/L BDCM and DMSO exposure groups) in the female medaka, but none in male medaka. The male:female ratios were skewed towards males suggesting possible androgenic or anti-estrogenic effects of BDCM. Exposure to

BDCM resulted in a dose-dependent increase in the proportion of males. Immature testis and ovaries, found in all concentrations including controls, are under evaluation. Other pathologic observations were granulomas and cystic ovaries, and granulomas in the testis with no apparent differences between exposed and control groups. The medaka embryo exposure protocol is useful for screening for endocrine disruption/reproductive toxicity.

C.2 BDCM

Effects of Defined Mixtures of Trihalomethanes and Haloacetic Acids on Pregnancy Maintenance and Eye Development in F344 Rats.

Narotsky M. G., Best D. S., McDonald A., Myers E. A., Hunter E. S. d. and Simmons J. E. Birth Defects Research. Part A, Clinical and Molecular Teratology 2006 76(5):384.

Although disinfection of drinking water is important for control of microbial contamination, it results in the formation of hundreds of disinfection by-products (DBPs). The most prevalent DBPs are trihalomethanes (THMs; chloroform, bromodichloromethane, chlorodibromomethane, bromoform) and haloacetic acids (HAAs; chloroacetic, dichloroacetic, trichloroacetic, bromoacetic, and dibromoacetic acid). THMs and HAAs are regulated in drinking water at 80 and 60 ug/L, respectively. In rats, THMs have been shown to cause pregnancy loss (i.e., full-litter resorption, an all-or-none effect). HAAs have been shown to cause eye defects and partial-litter, as well as full-litter, resorption (i.e., not an all-or-none effect). Here, we assessed the combined toxicity of these DBPs. Rats were treated with mixtures of four THMs (THM4), five BAAs (HAA5), or nine DBPs (DBP9; THM4 + HAA5). Chemical proportions reflected those in tap water; e.g., in DBP9, molar percentages of the respective chemicals were 30.0, 11.9, 7.0, 1.0, 1.7, 22.7, 15.9, 8.2, and 1.5. Mixtures, prepared in 10% Alkamuls[®]; EL-620, were administered daily to F344 rats by gavage on gestation days 6-20. Litters were examined on postnatal days 1 and 6. For the THM4 mixture, pregnancy loss was seen in 0/14, 0/25, 11/14 (79%), and 12/13 (92%) of the dams at 0, 307, 613, and 920 umol/kg, respectively. Pup weights were reduced at 613 and 920 umol/kg. Postnatal loss was increased at 920 umol/kg. For the HAA5 mixture, pregnancy loss was seen in 0/9, 0/15, 3/17 (18%), and 11/11 (100%) of the dams at 0, 308, 615, and 1231 umol/kg, respectively. Eye malformations (anophthalmia or microphthalmia) were seen in 0, 53%, and 79% of the live litters at 0, 308, and 615 umol/kg. Prenatal loss was unaffected in live litters. For the DBP9 mixture, pregnancy loss was seen in 0/18, 0/19, 6/17 (35%), and 7/8 (88%) of the dams at 0, 307, 615, and 1228 umol/kg, respectively. In live litters, prenatal loss was unaffected. Thus, THM4, HAA5, and DBP9 each caused pregnancy loss at ≥ 613 umol/kg; i.e., both HAAs and THMs contributed to DBP9-induced pregnancy loss. The presence of THMs in the full mixture, however, reduced the incidence of BAA-induced eye defects.

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Narotsky M. G., Best D. S., McDonald A., Myers E. A., Hunter E. S. and Simmons J. E. Birth Defects Research. Part A, Clinical and Molecular Teratology 2005 73(5):358.

Although disinfection of drinking water is vitally important for eliminating microbial contamination, it also causes formation of hundreds of disinfection by-products (DBPs). The most prevalent DBPs are trihalomethanes (THMs; chloroform, bromodichloromethane, chlorodibromomethane, bromoform) and haloacetic acids (HAAs; chloroacetic, dichloroacetic, trichloroacetic, bromoacetic, and dibromoacetic acids). Some epidemiology studies have shown an increased risk of spontaneous abortion associated with consumption of water with high

concentrations of THMs. We have previously shown that bromodichloromethane causes pregnancy loss, i.e., full-litter resorption, in F344 rats via a luteinizing hormone-mediated mechanism (low-effect level = 305 umol/kg). HAAs, however, have not been quantified in these epidemiology studies, nor have they been tested in the F344 strain for their effects during gestation. In this study, we tested mixtures of the four THMs (THM4) as well as mixtures of the THMs and HAAs (DBP9; THM4 HAA5). Chemical proportions mimicked those found in drinking water. The DBP9 mixture consisted of ~50% THM4 and ~50% HAA5. Mixtures, prepared in 10% Alkamuls® EL- 620, were administered to F344 rats by gavage on gestation days 6-20. Litters were examined on postnatal days 1 and 6. For the THM4 mixtures, pregnancy loss was seen in 0/5, 0/10, 11/14 (79%), and 12/13 (92%) of the dams at 0, 307, 613, and 920 umol/kg, respectively. Among surviving litters, pup weights were reduced at 613 and 920 umol/kg. Postnatal loss was increased at 920 umol/kg. For the DBP9 mixtures, pregnancy loss was seen in 0/18, 0/19, 6/17 (35%), and 7/8 (88%) of the dams at 0, 307, 615, and 1228 umol/kg, respectively; no effects on pup weight or postnatal mortality were observed. It is noteworthy that all dams maintained pregnancy at 307 umol THM4/kg; however, the addition of HAA5 to this mixture (i.e., 307 umol THM4/kg 308 umol HAA5/kg 615 umol DBP9/ kg) resulted in pregnancy loss. This finding suggests that either HAAs cause pregnancy loss in F344 rats, or that HAAs potentiate the THMs’ disruptive effect on pregnancy. We plan to evaluate HAAs for their effects on pregnancy in the F344 rat.

Animal models for studying miscarriage: illustration with study of drinking water disinfection byproducts.

Narotsky M. G. and Laffan S. B.

Birth Defects Research. Part A, Clinical and Molecular Teratology 2004 70(5):283.

No single animal model is ideal for investigating spontaneous abortion. However, different species (e.g., rat, mouse, monkey) do offer research opportunities that can provide insights into possible causes of miscarriage. In the F344 rat, several chemicals [e.g., trichloroethylene, atrazine, bromodichloromethane (BDCM)] have been shown to cause pregnancy loss, i.e., full-litter resorption (FLR). BDCM, a drinking water disinfection byproduct, is of particular interest because of epidemiological evidence associating it with spontaneous abortion. In the rat, BDCM’s disruption of pregnancy is an all-or-none effect in that surviving litters appear to be unaffected. The F344 strain is particularly sensitive to this effect whereas the Long-Evans rat is moderately sensitive and the Sprague Dawley rat appears to be insensitive. Several lines of evidence indicate that BDCM disrupts pregnancy in the rat by a luteinizing hormone (LH)-mediated mode of action: 1) BDCM’s effect requires exposure during the LH-dependent period of pregnancy. 2) BDCM decreases serum levels of LH (and progesterone) in rats with FLR. 3) Treatment with hCG, an LH agonist, rescues BDCM-exposed pregnancies from FLR. 4) Corpora lutea cultured in BDCM are less responsive to hCG stimulation in vitro. Given that LH and hCG bind to the same luteal receptor, responsiveness to LH in rats is analogous to responsiveness to hCG in humans. Regarding LH secretion, if BDCM disrupts LH secretion in the rat via altered GnRH signaling, this could be relevant to human pregnancy in that placental secretion of hCG is also regulated by GnRH. Indeed, recent data demonstrate that BDCM inhibits human placental trophoblast differentiation and hCG secretion in vitro. Thus, animal

models may give important insights into the causes of miscarriage, and offer guidance for further research.

Effects of bromodichloromethane (BDCM) on ex vivo luteal function in the pregnant F344 rat.

Bielmeier S. R., Murr A. S., Best D. S., Goldman J. M. and Narotsky M. G.
Toxicologist 2003 72(S-1):26-7.

We have reported that BDCM, a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption, in F344 rats when treated on gestation day (GD) 6-10, encompassing the luteinizing hormone (LH)-dependent period. BDCM-induced pregnancy loss was associated with reductions in serum progesterone (P) and corresponding decreases in LH on GD 10, suggesting BDCM disrupts the maternal hypothalamic-pituitary-gonadal axis. These and other data indicate that BDCM affects the hypothalamus or pituitary gland; however, an effect on luteal responsiveness to LH had not been definitively excluded. To address this data gap, we used an ex vivo approach to assess luteal function. Dams were dosed by gavage on GD 6-9 (plug day = GD 0) at 0 or 100 mg/kg/d (n = 11, 12). One hour after the GD-9 dose, rats were sacrificed, blood was collected and corpora lutea (CL) were incubated with or without hCG, an LH agonist, to stimulate P secretion. During the 24 h incubation, media were periodically sampled for hormone analysis by dissociation enhanced lanthanide fluorescent immunoassay (DELFIATM). Luteal responsiveness was unaffected; both groups displayed a 2.4-fold increase in P secretion in response to hCG challenge. Paradoxically, the BDCM-exposed CL showed greater than 2-fold increases in P secretion ex vivo regardless of the presence of hCG; whereas the same animals, i.e., the CL donors, had decreased serum P and LH levels in vivo. It is unclear if this 'rebound' effect reflects the removal of the CL from a possible direct inhibitory influence of BDCM, or a response to the diminished LH stimulation in vivo. Regardless, the lack of effect on luteal responsiveness is further evidence that BDCM-induced pregnancy loss in the rat is due to reduced pituitary LH secretion.

Dose additivity of atrazine and bromodichloromethane in causing pregnancy loss in F344 rats.

Narotsky M. G., Best D. S., Bielmeier S. R. and Cooper R. L.
Toxicologist 2003 72(S-1):77.

Atrazine (ATRZ), a widely used herbicide, and bromodichloromethane (BDCM), a disinfection by-product found in drinking water, have both been shown to cause pregnancy loss, i.e., full-litter resorption (FLR), in F344 rats. Although chemically quite different, both ATRZ and BDCM have similar modes of action; ATRZ- and BDCM-induced pregnancy loss are associated with reduced levels of luteinizing hormone (LH) and progesterone during the LH-dependent period of gestation. ATRZ and BDCM co-exist in drinking water; thus, we sought to evaluate their cumulative effect on pregnancy maintenance using a dose-additivity model. Each agent was administered alone at near-threshold doses and in combination at one-half of the near-threshold doses. If synergistic, the two sub-threshold doses would combine to cause a greater-than-threshold response. Both agents were administered by gavage (BDCM in 10% alkamuls EL-620,

then ATRZ in 1% methylcellulose) on gestation days 6-10. Each agent was administered alone at 40 mg/kg/d (LOEL = 50 mg/kg); whereas the two were administered together at 20 mg/kg/d each. Dams were allowed to deliver and litters were examined on postnatal days 1 and 6. Uteri of nonparous females were stained with 10% ammonium sulfide to detect FLR. Alone or in combination, the agents were maternally toxic, causing weight loss after the first dose. As expected, 40 mg/kg of each chemical was a near-threshold dose for causing FLR; low rates (5-6%; 1 affected of 18-19 dams) were seen for each chemical alone. In combination, no FLR was seen (n = 19). Thus, in this study, ATRZ and BDCM clearly lacked synergy in their ability to cause pregnancy loss in F344 rats. Although we did not assess the possibility of antagonism, these results are consistent with the default risk-assessment assumption of dose additivity for agents with the same mechanism.

Strain comparison of endocrine response in rats to bromodichloromethane (BDCM) during pregnancy.

Bielmeier S. R., Best D. S. and Narotsky M. G.
Toxicologist 2002 66(1-S):374.

Previously, we reported that BDCM, a drinking water disinfection by-product, causes pregnancy loss, i.e. full-litter resorption (FLR) in F344 rats when treated during the luteinizing hormone (LH)-dependent period. We also found that Sprague-Dawley (SD) rats were unaffected at even higher doses. Here, we examined the effects of BDCM on serum progesterone and LH levels during pregnancy in both strains. BDCM, in 10% emulphor, was dosed by gavage on gestational days (GD) 6-10 (plug day = GD 0) at 75 mg/kg/d in the F344 rat, and 100 or 200 mg/kg/d in the SD rat. Blood samples were collected from the lateral tail vein once daily on GD 6-11; serum progesterone and LH were measured using DELFIA[®]. The rats were allowed to deliver and pups were examined postnatally. FLR was confirmed by staining uterine resorption sites with 10% ammonium sulfide. Unlike the F344 rats, the SD rats maintained their litters and progesterone levels; although, similar to the F344 rats, they did display decreased LH levels. F344 rats had an 88% (7/8) incidence of FLR at 75 mg/kg/d, whereas SD rats had 0% (0/10, 0/9) incidences at both 100 and 200 mg/kg/d. BDCM-treated F344 dams with FLR had reduced progesterone and LH levels on GD 10; mean +/- SE LH levels were 0.07 +/- 0.04 vs. 0.15 +/- 0.02 ng/mL for controls. In contrast, the SD rats treated at 200 mg/kg/d did not show a decrease in serum progesterone levels on GD 10; however, serum LH levels were significantly decreased from controls (0.24 +/- 0.06 vs. 0.49 +/- 0.06 ng/mL). Importantly, control serum LH values were significantly higher in SD than F344 rats (p less than 0.0001). Thus, these dramatic strain differences in baseline LH levels and susceptibility to BDCM-induced pregnancy loss exemplify the importance of strain as a consideration when assessing reproductive toxicity.

Developmental consequences of exposure to disinfection by-products in animal models.

Hunter E. S.
Toxicologist 2002 66(1-S):331.

Disinfection by-products (DBPs) have been associated with a variety of adverse developmental outcomes in epidemiology studies including cardiac defects, neural tube defects, still births, and

spontaneous abortions. In toxicology studies, the trihalomethanes (THMs) do not produce high rates of dysmorphogenesis, but selected THMs do cause pregnancy loss, i.e. full-litter resorptions (FLR) in rats. Serum luteinizing hormone (LH) is diminished when bromodichloromethane (BDCM) is administered to Fischer 344 rats during the LH-dependent period of pregnancy maintenance suggesting that perturbation of the endocrine axis may be responsible for FLR. The possibility that BDCM compromises LH responsiveness is also being explored as this has more relevance to human pregnancy maintenance. Among the haloacetic acid (HA) class of DBPs, trichloroacetate (TCA) and dichloroacetate (DCA) each induce heart, eye and kidney defects in rats. Dibromoacetate (DBA) produces small/absent kidneys in mice, eye defects in rats and skeletal abnormalities in both species. Bromochloroacetate (BCA) exposure results in decreased fetal implants and live fetuses. In the rat, BCA reduced day 6 pup weights, and DBA induced pre- and postnatal loss and reduced pup weight on PND 1 and 6. When compared in mouse whole embryo culture (WEC) the relative potency of the HAs are BCA greater than DBA greater than DCA. Dose additivity of DCA, DBA and BCA was observed in rat WEC. Haloacetoneitriles, another class of DBPs, induce FLR (dichloroacetoneitrile (DCAN) and trichloroacetoneitrile (TCAN)), decrease birth weight (chloroacetoneitrile, DCAN, TCAN, bromochloroacetoneitrile BCAN), dibromoacetoneitrile (DBAN)), decreased postnatal weight gain (DCAN, BCAN, DBAN) and increased postnatal mortality (DCAN, TCAN). While most DBPs produce effects on development and neonatal growth, many are only effective at doses that produce maternal toxicity. In vitro, embryos show induction of cell death with little concurrent effect on the cell cycle. Mechanistic studies are focused on HA-induced alteration in signal transduction pathways.

Effects on the gonadal development of medaka (*Oryzias latipes*) exposed as embryos to bromodichloromethane.

Thiyagarajah A., Parker S., Gennings C., Teuschler L. E., Conerly O. and Hartley W. R. Toxicologist 2002 66(1-S):374-5.

The purpose of this study is to determine the toxic effects of bromodichloromethane (BDCM) on the developing gonad of medaka. BDCM is one of the trihalomethanes, a disinfection by-product found in the drinking water with developmental/reproductive toxicity concerns expressed in epidemiological studies. Medaka embryos at early high blastula stage were exposed to 10 or 25 mg/L BDCM for 10 days, and transferred to contaminant-free embryo rearing medium until hatched. Upon hatching, medaka fry were grown-out in spring water for 6 and 12 months. Two controls (embryo-rearing solution and DMSO carrier) were used. Fish were sampled at 6 and 12-month post-exposure and processed for histological evaluation. The evaluated endpoints, indicative of endocrine disruption or other reproductive toxicities were: hermaphroditism; immature gonads; ectopic gonads; male:female ratio; and other pathology. Hermaphroditism was not observed. There were two cases of ectopic gonads (10 mg/L BDCM and DMSO exposure groups) in the female medaka, but none in male medaka. The male:female ratios were skewed towards males suggesting possible androgenic or anti-estrogenic effects of BDCM. Exposure to BDCM resulted in a dose-dependent increase in the proportion of males. Immature testis and ovaries, found in all concentrations including controls, are under evaluation. Other pathologic observations were granulomas and cystic ovaries, and granulomas in the testis with no apparent

differences between exposed and control groups. The medaka embryo exposure protocol is useful for screening for endocrine disruption/reproductive toxicity.

Full-litter resorptions caused by low-molecular weight halocarbons in F-344 rats.

Narotsky M. G., Hamby B. T., Mitchell D. S. and Kavlock R. J.
Teratology 1992 45(5):472-3.

In the evaluation of the developmental toxicity of a variety of chemicals present either at Superfund sites or in drinking water following disinfection, we observed a distinctive toxic response with low-molecular weight halocarbons. F-344 rats were gavaged on gestation days (GD) 6-15 with trichloroethylene (TCE), tetrachloroethylene (TetCE), carbon tetrachloride (CCl₄), dichloromethane (DCM), bromoform (BF), or bromodichloromethane (BDCM) in corn oil. Each was tested with at least two dose levels plus a concurrent control group; the high dose was selected to cause maternal toxicity. The dams were allowed to deliver and their litters were examined post-natally. For five of the six chemicals, dose-related incidences of full-litter resorptions were a prominent developmental effect. This effect was generally seen in treatment groups where dams lost an average of 3-15 g over GD 6-8; control dams gained 2-4 g over this period. For DCM, which did not elicit the effect, a smaller change in maternal weight was evident. Within group comparisons of dams resorbing and maintaining their litters suggested only a weak relationship between maternal weight change and whole-litter loss. For TCE, TetCE, and CCl₄, the resorption sites were generally visible only after staining with ammonium sulfide, suggesting very early embryonic death. For BF and BDCM, however, practically all affected litters had at least some resorption sites visible prior to staining. The all-or-none nature of this effect suggests a maternally mediated response. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

C.3 DBCM

No abstracts were identified in this category.

C.4 Bromoform

Chronic pathological effects from exposure of Japanese medaka (*Oryzias latipes*) embryos to bromoform.

Thiyagarajah A., Teuschler L. K., Lipscomb J. C., Gennings C. and Hartley W. R.
Toxicologist 2000 54(1):185.

The purpose of this study was to determine the chronic toxicity of bromoform in an alternative test species, Japanese medaka (*Oryzias latipes*). In this study, we exposed medaka embryos at 64-cell stage (early high blastula) to 0, 5, 10, 25 and 50 mg bromoform/L (nominal concentrations) and DMSO (solvent carrier) for 10 days. The embryos then transferred to clean

embryo-rearing solution until they hatch, followed by six to twelve month grow-out in spring water. At 6 and 12 months post hatch, up to 60 fish were killed by overdose of anesthesia, fixed in 10% neutral buffered formalin, and evaluated histologically. Immature gonads and cystic ovaries were found in all groups, but the incidence was higher in bromoform-exposed fish. The incidence of thyroid follicular hyperplasia was higher at twelve months in bromoform-exposed fish. A low incidence of a variety of renal degenerative lesions occurred in both control and bromoform-exposed fish. However, the incidence of renal cystic tubules was higher in bromoform-exposed fish. A low incidence of degenerative and non-neoplastic proliferative lesions were observed in the livers of both control and bromoform-exposed fish. There was an increased incidence of hepatic cysts in the six-month old medaka exposed to bromoform. Dose-response of histopathological endpoints will be discussed. Critical endpoints from this study will be compared to the chronic toxicity of bromoform in rodent risk assessment models.

Bromoform requires a longer exposure period than carbon tetrachloride to induce pregnancy loss in F-344 rats.

Narotsky M. G., Hamby B. T., Mitchell D. S. and Kavlock R. J.
Toxicologist 1993 13(1):255.

Bromoform (BF) and carbon tetrachloride (CCl₄), among other low-molecular weight halocarbons, have been shown to induce full-litter resorption (FLR) when administered by gavage to F-344 rats throughout organogenesis. To compare their effects after a shorter exposure period, BF and CCl₄ were gavaged in corn oil at 200 and 150 mg/kg/day, respectively, on gestation days (GD) 6-7 or 6-15. Cesarean sections were performed on GD 20 and all uteri were stained with 10% ammonium sulfide to detect sites of early resorption. When administered on GD 6-15, both compounds caused FLR in at least 9/10 dams. When administered on GD 6-7, however, CCl₄ caused FLR in 8/10 dams whereas BF caused FLR in only 1/10 dams. Resorption rates were unaffected in surviving litters. Both halocarbons caused maternal weight loss during GD 6-8. This effect was slightly, but significantly, greater in the CCl₄-treated dams. The near absence of FLR for the 2-day BF exposure indicates that pregnancy loss requires prolonged exposure and occurs after GD 7. However, the single case of FLR suggests a reduced risk with decreasing exposure duration, rather than a discrete critical period for the effect. For CCl₄, the 2-day exposure was sufficient to induce FLR, but the embryonic stage affected remains to be determined.

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Narotsky M. G., Hamby B. T., Mitchell D. S. and Kavlock R. J.
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In the evaluation of the developmental toxicity of a variety of chemicals present either at Superfund sites or in drinking water following disinfection, we observed a distinctive toxic response with low-molecular weight halocarbons. F-344 rats were gavaged on gestation days (GD) 6-15 with trichloroethylene (TCE), tetrachloroethylene (TetCE), carbon tetrachloride (CCl₄), dichloromethane (DCM), bromoform (BF), or bromodichloromethane (BDCM) in corn oil. Each was tested with at least two dose levels plus a concurrent control group; the high dose

was selected to cause maternal toxicity. The dams were allowed to deliver and their litters were examined post-natally. For five of the six chemicals, dose-related incidences of full-litter resorptions were a prominent developmental effect. This effect was generally seen in treatment groups where dams lost an average of 3-15 g over GD 6-8; control dams gained 2-4 g over this period. For DCM, which did not elicit the effect, a smaller change in maternal weight was evident. Within group comparisons of dams resorbing and maintaining their litters suggested only a weak relationship between maternal weight change and whole-litter loss. For TCE, TetCE, and CCl4, the resorption sites were generally visible only after staining with ammonium sulfide, suggesting very early embryonic death. For BF and BDCM, however, practically all affected litters had at least some resorption sites visible prior to staining. The all-or-none nature of this effect suggests a maternally mediated response. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

C.5 Chloroform

Fetal Exposure To Water Disinfection By-Products Alters Postnatal Growth And Glucose Homeostasis.

Lim G. E., Stals S. I., Foster W. G., Petrik J. J. and Holloway A. C.

Journal of the Society for Gynecologic Investigation 2004 11(2 Suppl).

Introduction: Epidemiological studies reveal that in utero insults resulting in impaired fetal growth have long-term consequences for postnatal health including an increased risk of type 2 diabetes as adults. It has been well documented that chlorination of drinking water results in the formation of trihalomethanes (THM). Epidemiological studies have shown that exposure to THMs during pregnancy may result in adverse developmental effects including fetal growth restriction. Given the importance of birthweight as a predictor of adult health in human populations, and the association between in utero exposure to THMs and intrauterine growth restriction, we therefore propose that fetal exposure to THMs may have life-long health consequences, which have not been previously identified. Objective: In this study, we examined the effect of in utero exposure to chloroform, the most predominate THM, on fetal and postnatal growth, and postnatal glucose homeostasis. Methods: Female nulliparous Wistar rats were given chloroform in their drinking water during pregnancy through to weaning at concentrations of 0ug/L, 75ug/L, and 150ug/L, which are representative of levels found in North American public water supplies. Dams were allowed to deliver normally, and the litters were culled to three males which remained with the dam until weaning. Serum and pancreas tissue were collected from the remaining animals. At 4 weeks of age, pups were challenged with an oral glucose load to evaluate serum glucose, and fasting serum glucose was measured at 15 weeks of age. Results: Birth weight in animals exposed to chloroform in utero was reduced relative to control animals but this effect did not reach significance. At weaning, body weights of both chloroform treatment groups were significantly lower than control animals. This effect of chloroform to inhibit postnatal growth persisted in the 75ug/l group until 22 weeks of age, and until 8 weeks of age body in the 150ug/l group. At PND1, serum glucose concentrations were elevated in pups born to chloroform exposed dams. This effect had disappeared by 4 weeks of age, and there was no

difference between groups in the response to an oral glucose load at 4 weeks of age. At 15 weeks of age however, fasting serum glucose concentrations were significantly lower in pups from the 75ug/L treatment group. Conclusion: These data show for the first time that fetal and neonatal exposure to chloroform at concentrations found in North American public drinking water supplies can inhibit postnatal growth and alter glucose homeostasis in juvenile animals. We propose that THM exposure in utero and postnatally may have a profound effect on children's health which has not been fully explored.

Long-term effects on male reproduction of early exposure to common chemical contaminants in drinking water.

Veeramachaneni D. N., Palmer J. S. and Amann R. P.
Human Reproduction 2001 16(5):979-87.

We evaluated sequelae to early exposure of male rabbits to drinking water containing chemicals typical of ground water near hazardous waste sites. The mixture (p.p.m. at 1x) was 7.75 arsenic, 1.75 chromium, 9.25 lead, 12.5 benzene, 3.75 chloroform, 8.5 phenol and 9.5 trichloroethylene. Dutch-Belted does received mixture at 0x (deionized water; control), 1x or 3x as drinking water from day 20 pregnancy through weaning. Exposure of individual males (7-9/treatment) continued until 15 weeks (adolescence); then, all males received deionized water. At 57-61 weeks of age, ejaculatory capability and seminal, testicular, epididymal and endocrine characteristics were evaluated. At 10 opportunities with a female teaser, all seven control males ejaculated every time, but 12 of the 17 treated males failed to express interest, achieve erection and/or ejaculate on one to five occasions; four of the 12 accomplished ejaculation with a second male teaser. Total spermatozoa/ejaculate and daily sperm production were unaffected. However, treatment caused ($P < 0.03$) acrosomal dysgenesis and nuclear malformations. Baseline serum concentrations of LH were lower, but with borderline significance ($P = 0.05$). Testosterone secretion after exogenous human chorionic gonadotrophin ($P < 0.04$) was low. Thus, even at 45 weeks after last exposure to drinking water pollutants, mating desire/ability, sperm quality, and Leydig cell function were subnormal.