Original Contribution

Water Disinfection By-Products and Prelabor Rupture of Membranes

Sarah J. Joyce¹, Angus Cook¹, John Newnham², Michael Brenters², Chantal Ferguson¹, and Philip Weinstein¹

¹ School of Population Health, University of Western Australia, Crawley, Western Australia, Australia.
² School of Women’s and Infants’ Health, University of Western Australia, King Edward Memorial Hospital for Women, Subiaco, Western Australia, Australia.

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The causes of term prelabor rupture of membranes (term PROM) remain poorly defined. The authors conducted a record-based prevalence study to explore a possible relation between disinfection by-products in drinking water and term PROM in an Australian community with spatially variable trihalomethane and nitrate levels. A multilevel statistical model was used to examine the relation between factors operating at the levels of the individual, district, and water distribution zone and the prevalence of PROM at term among 16,229 women in Perth, Western Australia (2002–2004). Adjusted odds ratios for term PROM increased with increasing tertiles of nitrate exposure (moderate exposure: odds ratio = 1.23, 95% confidence interval: 1.03, 1.52; high exposure: odds ratio = 1.47, 95% confidence interval: 1.20, 1.79), but there was no significant relation with exposure to trihalomethanes. This study raises the possibility that water contaminants may promote the development of PROM at term.

disinfection; fetal membranes, premature rupture; nitrates; nitrosamines; reactive oxygen species; trihalomethanes; water purification

Abbreviations: CI, confidence interval; MMP, matrix metalloproteinase; OR, odds ratio; PROM, prelabor rupture of membranes; SEIFA, Socio-Economic Indexes for Areas.

Prelabor rupture of membranes

Prelabor rupture of membranes (PROM) is defined as the rupture of membranes before the onset of labor (1). At term (pregnancies of 37 or more weeks’ duration), approximately 8–10 percent of pregnant women experience PROM (2), and the condition is associated with an increased risk of intrapartum infection, umbilical cord prolapse, fetal distress, umbilical cord compression, and placental abruption (3, 4). Such outcomes often require obstetric interventions, including emergency cesarean sections and instrumental vaginal deliveries, which carry their own risks (5–7). Term PROM is a leading cause of early hospital admission and clinical monitoring among pregnant women, and hence it incurs significant financial and human costs (8).

Despite the high prevalence of term PROM, the biologic mechanisms of the condition and the risk factors for its development remain largely unclear. Recent studies have shown that at the molecular level, term PROM may result from diminished collagen synthesis, altered collagen structure, and accelerated collagen degradation within the extracellular matrix of the fetal membranes (1, 9, 10). Molecular and genetic research has focused on the role of matrix metalloproteinases (MMPs), a group of enzymes which are capable of breaking down the collagenous extracellular matrix of the fetal membranes (11–13). Studies have shown enzyme activity to be increased in the membranes of women with PROM, with MMP-9 being particularly active (14–16). There is also emerging evidence that reactive oxygen species are specifically capable of damaging collagen (via the...
MMP-9 enzyme) in the fetal membranes, which could in turn cause PROM. Recent in vitro studies have shown the reactive oxygen species hydrogen peroxide and superoxide anions inducing MMP-9 activity in amniochorionic membranes (17, 18). Correspondingly, in in vitro studies where segments of fetal membranes were treated with antioxidants, MMP-9 activity was significantly suppressed (18, 19).

**Disinfection by-products**

Although the benefits of drinking water disinfection are acknowledged, there is growing concern over the health risks associated with exposure to disinfection by-products. Disinfection by-products are a group of compounds formed when disinfectants, including chloride, react with naturally occurring substances (e.g., organic products of decaying vegetation) present in the source water (20). In 1974, trihalomethanes became the first class of disinfection by-products to be identified, and a significant body of research currently exists on their occurrence and toxicity. More recently, nitrogenated disinfection by-products, including haloacetonitriles, halonitromethanes, and N-nitroamines (e.g., N-nitrosodimethylamine), have been recognized, and there is emerging evidence that these may be more toxic than the more commonly measured halogenated forms, such as trihalomethanes (21–23). Epidemiologic studies also provide moderate support for associations of disinfection by-products with adverse pregnancy outcomes, although again the majority of these focus on the routinely measured halogenated compounds (24–26).

Production of reactive oxygen species occurs in response to various stimuli, including infections, but evidence suggests that other agents may also play a role. Chemical toxicants, including disinfection by-products and nitrates/nitrites, may promote the formation of reactive oxygen species and independently stimulate enzyme activity. Several disinfection by-products and nitrates/nitrites commonly found in drinking water have been associated with the formation of reactive oxygen species in macrophage cell lines (27), fish (28), and rat gastric mucosal cells (29). Chemical stimuli may therefore act to trigger inflammation and release of free radicals, thereby inducing MMP activity, collagen degradation, and ultimately rupture of the placental membranes.

**Water supply in Perth, Western Australia**

In Western Australia, the Perth metropolitan region is made up of 24 separate water distribution zones. Each distribution zone has different concentrations of disinfection by-products because of varying water sources (groundwater vs. surface water), geologic formations, and seasonal dynamics that differentially affect ground and surface water sources. The local water utility, the Water Corporation of Western Australia, regularly monitors the water quality in each distribution zone, and water contaminant concentrations for each area are publicly available in its annual report. A distinct gradient exists in the Perth region, with some areas receiving substantially lower concentrations of different compounds.

Trihalomethanes and nitrates are two compounds routinely tested for by the water utility. Nitrate is an inorganic compound that occurs naturally in drinking water sources due to leaching from mineral deposits, and it may also arise from particular land uses (e.g., application of agricultural fertilizers). In Western Australia, elevated levels of nitrate mainly develop as a result of natural processes of plant decay underground that has occurred over geologic time (30). As a result, nitrate occurs mainly in the groundwater aquifers which supply approximately 50 percent of the metropolitan region. High levels of nitrogen in these waters may react with disinfectants to form nitrogenated forms of disinfection by-products, which have been postulated to exert toxicologic effects.

Despite the high prevalence of term PROM, the risk factors associated with this pregnancy outcome remain largely unclear. A range of water disinfection by-products, including nitrogen-based forms (such as haloacetonitriles), have been linked with other adverse birth outcomes and could explain PROM through an oxidative stress mechanism (31, 32). To address this gap in the literature, we used multilevel modeling to examine the association between disinfection by-products and PROM at term among pregnant women residing in Perth, Western Australia, during 2002–2004.

**MATERIALS AND METHODS**

**Study design and population**

A record-based prevalence study design was used. All primigravid Caucasian women who had given birth to a single newborn in Western Australia during 2002–2004 were included in the study. We selected this restricted study population in order to minimize differences in risk profiles, since the frequency of term PROM may vary with multiparity and ethnicity.

**Individual-level data**

De-identified data were obtained for the study population from the Western Australia Midwives’ Notification System for the period 2002–2004 inclusive. The Midwives’ Notification System is a register of all home and hospital births that have occurred in Western Australia since 1980. The system provided information at an individual level on a range of variables, including maternal age, smoking, and pregnancy complications. The outcome of interest was defined as PROM at term (37 weeks’ gestation or later) as recorded by the midwife. We conducted a small interobserver reliability study and concluded that the data provided by the Midwives’ Notification System were reliable (κ = 88.6 percent).

Residential address is also recorded by the Midwives’ Notification System at the time of birth. Using purpose-built address parsing and matching software, we assigned each study subject a collection district and water distribution zone code. Collection districts are spatially defined by the Australian Bureau of Statistics and comprise, on average, 220 households (33).

**District-level data**

The assigned residential collection district for each individual was also allocated a value from the Australian Bureau
Distribution zone-level data

Water distribution zones are spatially defined by the local water utility (35) and are routinely monitored for drinking water contaminants, including trihalomethanes and nitrates. Using ArcGIS, version 9.0 (ESRI, Redlands, California), we generated a map detailing the spatial boundaries of the 24 water distribution zones in Perth and overlaid it onto a map of collection district boundaries in the same region to determine which collection districts were serviced by each water distribution zone. We extracted mean water contaminant measurements for each distribution zone during the time period 2002–2004 from the Water Corporation of Western Australia’s historical database and used them to classify distribution zones as low-, medium-, or high-exposure areas for both trihalomethanes and nitrates.

In order to validate the use of a mean concentration for the entirety of each water distribution zone, we conducted an independent sampling program during 2006–2007. Additional water samples were obtained from five locations (north, south, east, west, and central) within six of the 24 water distribution zones in Perth. Distribution zones were chosen to maximize between-site variation for a number of selection criteria, including area size, population size, and water source (groundwater, surface water, or mixed). Each location was sampled twice over a period of 3 months for evaluation of reliability. Samples were collected from publicly accessible taps, including bathrooms at shopping centers and gasoline (petrol) stations and water fountains in public parks, and from locations that would be frequently used, thereby limiting the storage time of water in delivery pipe networks (a factor which may influence concentration values). Taps at each location were flushed for a period of 2 minutes prior to collection to ensure consistency between samples, as initial water flow may not be representative. All samples were stored on ice for preservation in an enclosed insulated carrier and were collected within an 8-hour period on each sample day.

Samples taken for trihalomethane measurements were collected in 40-ml glass vials, each dosed with the quenching agent sodium thiosulfate, to prevent further trihalomethane formation once the samples had been collected. During each collection, vials were filled to the maximum level in order to limit headspace and thus prevent air bubbles upon sealing and subsequent loss of trihalomethanes due to volatilization. Trihalomethane analysis was performed using the “purge and trap” technique for sample concentration and delivery, followed by gas chromatography with mass spectrometric detection. Laboratory reagent blanks and field duplicates were used, as well as an internal standard to correct for slight differences in temperatures and trapping conditions throughout the run. Samples taken for nitrate measurements were collected in 250-ml high-density polyethylene containers and measured for both nitrate and total nitrogen using the flow injection analysis method.

Statistical analysis

Because of the hierarchical nature of our data, a random intercept multilevel model was used to estimate the odds of PROM as a function of individual, district, and distribution zone characteristics. The software package MLwiN, version 2.0, was used (36). Adjusted odds ratios and 95 percent confidence intervals were derived on the basis of trihalomethane and nitrate exposure categories.

RESULTS

Perth water supply

Water distribution zones were classified as low-, medium-, or high-exposure areas based on their mean concentration over the period 2002–2004. Three exposure tertiles were created using the concentration cutoffs indicated in table 1. In order to select appropriate exposure category cutoffs, we generated distributional graphs illustrating the proportion of distribution zones (fraction of the data) within each quantile of trihalomethane and nitrate concentration. We also assessed the contaminant levels within each assigned exposure category using analysis of variance. The mean square errors and F test value indicated highly significant between-group differences and relatively little within-group variability for the specified category cutoff points for total trihalomethanes ($F = 69.70; p < 0.0001$) and nitrates ($F = 15.49; p < 0.0001$).

Independent sampling of six water distribution zones was conducted with water samples taken from five locations,
each representing the north, south, east, west, or central region of each water distribution zone, to facilitate the assessment of spatial variation of trihalomethane and nitrate concentrations within distribution zones. Generally, variation within each distribution zone was found to be limited, with the exception of two distribution zones that featured several outliers (Foothills and South Perth), both served by a mixture of surface and groundwater supplies.

Population characteristics

A total of 16,229 pregnant women from Perth for the period 2002–2004 were included in the study. These persons resided within 2,348 collection districts which were nested in 24 water distribution zones. The characteristics of this study population and their distribution across the three exposure tertiles are summarized in table 2. The prevalence of term PROM increased with increasing nitrate exposure, from 3.6 percent in the low nitrate exposure tertile to 5.1 percent in the high exposure category.

The relations between demographic characteristics and term PROM for the study population are presented in table 3. To some extent, PROM cases were more likely to be located in an area classified in one of the three lowest SEIFA categories—that is, they lived in relatively disadvantaged areas. There was also a slightly higher incidence of smoking during pregnancy among PROM cases, and they were more likely to fall within 20–30 years of age as compared with the entire study population. However, differences were negligible, and generally demographic features were similar.

Risk estimates

Table 4 presents the results of the final model for disinfection by-products and term PROM. In the final multilevel analysis, exposure to increasing concentrations of trihalomethanes in drinking water did not appear to be associated with an increase in PROM. Odds ratios, while slightly elevated, remained very close to unity. The crude odds ratios for PROM increased with each tertile of nitrate exposure. After adjustment, the risk of PROM remained significantly increased for persons residing in moderate- and high-exposure areas for nitrate in drinking water; odds ratios were 1.23 (95 percent confidence interval (CI): 1.03, 1.52) and 1.47 (95 percent CI: 1.20, 1.79), respectively. These estimates also reflect a dose-response trend, with an increasing risk estimate across increasing exposure tertiles.

We conducted additional analyses to determine whether risk estimates varied by socioeconomic status. Two strata were created above and below the population median SEIFA score, and multilevel-model analyses were rerun. Local (small-area) SEIFA values for each census district were still included to provide the highest possible spatial adjustment. For each unit increase in (log-transformed) nitrate levels, the odds ratio for the population below the median socioeconomic level (odds ratio (OR) = 1.15, 95 percent CI: 1.00, 1.33) was closely comparable to that for the population at or

**TABLE 2. Distribution of pregnant women across tertiles of exposure to water disinfection by-products, Perth, Western Australia, 2002–2004**

<table>
<thead>
<tr>
<th>Individual characteristic</th>
<th>Trihalomethane exposure</th>
<th>Nitrate exposure</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>No. of water distribution zones</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>No. of cases of term PROM*</td>
<td>120</td>
<td>283</td>
<td>284</td>
</tr>
<tr>
<td>No. of pregnant women</td>
<td>2,868</td>
<td>6,789</td>
<td>6,572</td>
</tr>
<tr>
<td>Prevalence of term PROM (%)</td>
<td>4.18</td>
<td>4.17</td>
<td>4.32</td>
</tr>
<tr>
<td>Average SEIFA* category†</td>
<td>3.6</td>
<td>3.7</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* PROM, prelabor rupture of membranes; SEIFA, Socio-Economic Indexes for Areas.
† 1 = lowest socioeconomic status/most deprived; 6 = highest socioeconomic status/least deprived.

**TABLE 3. Sociodemographic characteristics of pregnant women (n = 16,229) in Perth, Western Australia, 2002–2004**

<table>
<thead>
<tr>
<th>Individual characteristic</th>
<th>Cases of term PROM*</th>
<th>Study population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14–19</td>
<td>49</td>
<td>7.1</td>
</tr>
<tr>
<td>20–25</td>
<td>185</td>
<td>26.9</td>
</tr>
<tr>
<td>26–30</td>
<td>242</td>
<td>35.2</td>
</tr>
<tr>
<td>31–35</td>
<td>160</td>
<td>23.3</td>
</tr>
<tr>
<td>36–40</td>
<td>44</td>
<td>6.4</td>
</tr>
<tr>
<td>&gt;40</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Smoked during pregnancy</td>
<td>100</td>
<td>14.6</td>
</tr>
<tr>
<td>SEIFA* category†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>49</td>
<td>7.1</td>
</tr>
<tr>
<td>2</td>
<td>104</td>
<td>15.1</td>
</tr>
<tr>
<td>3</td>
<td>183</td>
<td>26.6</td>
</tr>
<tr>
<td>4</td>
<td>176</td>
<td>25.6</td>
</tr>
<tr>
<td>5</td>
<td>117</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>8.4</td>
</tr>
</tbody>
</table>

* PROM, prelabor rupture of membranes; SEIFA, Socio-Economic Indexes for Areas.
† 1 = lowest socioeconomic status/most deprived; 6 = highest socioeconomic status/least deprived.
above the median (OR = 1.14, 95 percent CI: 1.03, 1.27). Results for (log-transformed) trihalomethane levels were
dissimilar, although both remained nonsignificant (OR = 1.24 (95 percent CI: 0.79, 1.95) below the socioeconomic
classification. This resulted in little change in the
event that they produced erroneous ex-
concentrations of nitrates and trihalomethanes (Foothills and
stricting the subset of catchments with highly variable con-
median). We also conducted separate analyses by re-
the median). We also conducted separate analyses by re-
vented exposure classification. This resulted in little change in the estimates or the significance of the adjusted odds ratio esti-
unit increases in log-nitrate levels (OR = 1.14, 95 percent CI: 1.05, 1.25) or log-trihalomethane levels
(OR = 1.12, 95 percent CI: 0.76, 1.64).

**Supplementary analyses of preterm PROM**

Analyses of preterm PROM (PROM occurring at less than 37 weeks’ gestation) were conducted and compared with those for term PROM. Births associated with preterm PROM comprised 1.8 percent of total births as opposed to 4.2 percent with term PROM. In relation to other preterm births, the outcome of preterm PROM was not significantly related to
unit increases in log-trihalomethanes (adjusted OR = 0.96, 95 percent CI: 0.59, 1.55) or log-nitrates (OR = 1.05, 95 percent CI: 0.92, 1.19). Using total births (term and preterm combined) as the study population, preterm PROM again did not reveal any elevation in risk for log-trihalomethanes (adjusted OR = 0.97, 95 percent CI: 0.64, 1.46) or log-
nitrates (OR = 1.08, 95 percent CI: 0.97, 1.21). This condition and other associated birth outcomes (such as pre-
maturity) will form the basis for a separate analysis.

**DISCUSSION**

To our knowledge, this was the first study to assess the relative effects of environmental agents, specifically drink-
ing water disinfection by-products, on term PROM. Our

| TABLE 4. Crude and adjusted odds ratios for term prelabor rupture of membranes, by exposure to water disinfection by-products, Perth, Western Australia, 2002–2004 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Log-transformed trihalomethane level (µg/liter) | Crude OR† | 95% CI† | Adjusted OR† | 95% CI† |
| Nitrate exposure tertile | | | | |
| Low | 1.00 | 1.00 | 1.00 | 1.00 |
| Moderate | 1.03 | 0.76, 1.40 | 1.04 | 0.77, 1.41 |
| High | 1.04 | 0.77, 1.41 | 1.07 | 0.79, 1.45 |
| Log-transformed nitrate level (mg/liter) | 1.14* | 1.05, 1.24 | 1.14* | 1.05, 1.24 |
| Nitrate exposure tertile | | | | |
| Low | 1.00 | 1.00 | 1.00 | 1.00 |
| Moderate | 1.25* | 1.03, 1.52 | 1.23* | 1.03, 1.52 |
| High | 1.42* | 1.15, 1.76 | 1.47* | 1.20, 1.79 |

* p < 0.05.
† OR, odds ratio; CI, confidence interval.
‡ Adjusted for maternal age, smoking during pregnancy, and socioeconomic status.
over 100 diseases (38), and there is growing evidence that it plays a role in pathologic processes in female reproduction, including PROM. Reactive oxygen species have been identified as possible initiators of the enzyme activity which is responsible for PROM (39). Nitrate has been implicated in oxidative processes: When ingested, it is quickly converted to nitrite, which can easily be reduced to nitric oxide by commensal bacteria (40, 41), as well as systemically in blood and tissues (40). Exogenous sources of nitric oxide thereby contribute to whole-body production through a pathway that is independent of nitric oxide inhibitors (42), allowing for more distal and sustained oxidative effects (41). Inorganic nitrate from environmental sources—food, drinking water, and other sources—can thus be considered a storage pool for the systemic generation and activity of nitric oxide in the body (41).

Interpretation of these results must allow for several limitations in the study design. The most important source of error and possible bias in this study was the possible misclassification of exposure. Maternal exposure to disinfection by-products was estimated on the basis of average measures for the entire water distribution zone, and thus it did not account for individual variations in consumption and exposure to disinfection by-products within the exposure categories identified. Exposure misclassification may also have been introduced because of residential mobility during pregnancy or from exposures incurred outside the home. A survey of 78 pregnant women in Perth conducted by colleagues in 2004–2005 found that 15.6 percent of participants reported moving at some point during their pregnancy (Kim Chisholm, University of Western Australia, personal communication, 2007); this finding is similar to that of international studies (12–25 percent relocation of women during pregnancy) (43–45). No attempt was made to adjust for residential mobility in this study, because the critical exposure period during pregnancy for term PROM is unknown, and thus it would not have been possible to identify the time at which subjects should be reclassified. Furthermore, trends in migration whereby people consistently preferentially moved from high-trihalomethane/nitrate areas to low-trihalomethane/nitrate areas (or vice versa) were not expected, and thus any misclassification that did occur would have been nondifferential.

Unlike the other disinfection by-products, nitrate has several other exposure pathways (e.g., atmospheric pollution). Food is often the predominant source of nitrate for adults, contributing 30–95 percent of overall nitrate exposure, depending on the concentration of nitrate in a person’s drinking water (46–48). It was not possible within the constraints of this study to account for individual variations in diet, although again any misclassification would have tended to be nondifferential in nature.

The exposure estimates in this study are strengthened by the findings of independent sampling in the study area, which indicated that within-zone variability with respect to contaminant concentrations was much lower than between-zone variability—a characteristic which some researchers have suggested indicates that water zone mean values are a valid way of differentiating exposure to water contaminants between individuals (49). This study also had a large number of birth records and complete information on individual covariates. This almost total level of subject ascertainment limits the effect of selection biases. The restricted selection criteria ensured that the study population was homogeneous with respect to ethnicity, parity, and singular gestations. Use of data from the Midwives’ Notification System also enabled us to adjust for a number of potentially confounding variables, including maternal age, smoking, and socioeconomic status. In several multilevel studies (50–52), investigators have concluded that the social environment or “neighborhood effect” has an influence on individual health that is independent of individual factors. The results in table 2 indicate that socioeconomic factors are unlikely to account for the spatial associations seen with term PROM. In fact, the areas with the poorest water quality were in the group with marginally higher socioeconomic status, which is in fact contrary to the trend that would be expected if socioeconomic deprivation were the main explanatory factor. Indeed, an associated analysis of this population indicated an odds ratio of 0.93 (95 percent CI: 0.88, 0.99) for every unit increase in SEIFA category—that is, a 7 percent reduction in the odds of term PROM for each unit rise in socioeconomic status (data not shown).

In summary, these preliminary results suggest a relation between the development of term PROM and exposure to water disinfection by-products. It is uncertain whether nitrate per se is likely to be the agent of interest or whether this compound acts as an indicator for the formation of other nitrogenated compounds, such as halonitromethanes.

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Conflict of interest: none declared.

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