Birth outcomes and water: A multidisciplinary study

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Birth defects are a known cause of infant death in the United States. Nitrate and atrazine are widely used agrichemicals found in U.S. drinking water and frequently occur as mixtures. While studies explore the risk of adverse pregnancy outcomes associated with exposure to these agrichemicals as single compounds, we hypothesize the mixture is more toxic. Nitrate and atrazine can react in vivo to form N-nitrosodiazine (NNAT), a nitrosamine. Many nitrosamines are known or suspected teratogens. Avian embryos exposed to NNAT showed increased incidence of neural tube defects, microphthalmia, craniofacial hypoplasia, heart defects, gastrochisis and caudal regression compared to embryos exposed to nitrate or atrazine. Our objective is to further examine the risk of adverse health outcomes associated with exposure to nitrate and nitrosatable agrichemicals in drinking water. To date, Nebraska and NNAT have been the focus of our population and laboratory studies. Presented here are some preliminary findings illustrating the multidisciplinary nature of this type of research.

Outcome
Birth defect rates for each of the 93 Nebraska counties were obtained from Nebraska Department of Health and Human Services (2005-2014) and expressed as case rate per 10,000 live births.

Exposure
Contaminant data for nitrate and nitrosatable agrichemicals were obtained from the Quality-Assessed Agrichemical Contaminant for Nebraska Ground Water database (1977-2014).

- Well types include commercial, domestic, irrigation, public water system, and livestock.
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Statistical analysis
Percentage of wells positive for each agrichemical was calculated. A linear regression model was fitted for nitrate and NNAT in different well types to determine if there was a relationship between percent positive agrichemical wells and birth defect rates. All analyses were conducted using SAS version 9.4.

Results

Association between domestic wells and other well types.

<table>
<thead>
<tr>
<th>Percent Domestic Wells</th>
<th>Correlation Coefficient (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate Positive</td>
<td>0.77 (0.0000)</td>
</tr>
<tr>
<td>Public</td>
<td>0.59 (0.0001)</td>
</tr>
<tr>
<td>Commercial</td>
<td>0.61 (0.03)</td>
</tr>
<tr>
<td>Percent Domestic Wells</td>
<td></td>
</tr>
<tr>
<td>Atrazine Positive</td>
<td>0.32 (0.04)</td>
</tr>
<tr>
<td>Public</td>
<td>0.55 (&lt;0.0001)</td>
</tr>
<tr>
<td>Irrigation</td>
<td>0.45 (&lt;0.0001)</td>
</tr>
</tbody>
</table>

Linear regression between birth defect rates and percent of agrichemical positive wells.

<table>
<thead>
<tr>
<th>Agrichemical (%)</th>
<th>Shape</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any NNAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only Parent P0</td>
<td>3.12</td>
<td>0.02</td>
</tr>
<tr>
<td>Only Parent D0</td>
<td>3.92</td>
<td>0.03</td>
</tr>
<tr>
<td>Only Degradate P0</td>
<td>2.16</td>
<td>0.26</td>
</tr>
<tr>
<td>Nitrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrazine D</td>
<td>4.33</td>
<td>0.03</td>
</tr>
<tr>
<td>Nitrate D</td>
<td>-2.71</td>
<td>0.14</td>
</tr>
<tr>
<td>Atrazine P</td>
<td>3.32</td>
<td>0.02</td>
</tr>
<tr>
<td>Nitrate P+D</td>
<td>-6.37</td>
<td>0.02</td>
</tr>
<tr>
<td>Atrazine P+D</td>
<td>1.87</td>
<td>0.05</td>
</tr>
<tr>
<td>Nitrate+NNAT P+D</td>
<td>-2.45</td>
<td>0.19</td>
</tr>
<tr>
<td>Atrazine+NNAT P+D</td>
<td>6.44</td>
<td>0.002</td>
</tr>
<tr>
<td>Nitrate+NNAT P+D</td>
<td>5.73</td>
<td>0.03</td>
</tr>
</tbody>
</table>

References

Acknowledgments
Funding was provided by the University of Nebraska Collaborative Initiative and Daugherty Water for Food Global Institute. We also thank Colleen Steele for performing queries for well data from the database and Les Howard for mapping.

Discussion
- Majority of wells tested for agrichemicals were irrigation (62%) and domestic wells (23%).
- Sixty three percent of domestic wells tested positive for nitrate and 25% of public wells tested positive for atrazine.
- Nitrate-positive domestic wells were correlated to nitrate-positive livestock wells. Atrazine-positive domestic wells were correlated to atrazine-positive irrigation wells.
- This suggests that if a livestock well is contaminated with nitrate, a nearby domestic well could be contaminated if the water is from the same aquifer.
- If an irrigation well is contaminated with atrazine, a nearby domestic well could be contaminated if the water is from the same aquifer.
- The basis for a negative relationship between nitrate contaminated wells and birth defect rates is less apparent, but if nitrate induces miscarriage it reduces the incidence of birth defects because the fetus does not survive to delivery.
- The largest effect of agrichemicals on birth defect rates was found with nitrate + atrazine in public and domestic wells.

Conclusions and Limitations
- This study suggests the association between birth defect rates and wells positive for both nitrate and atrazine is stronger than with wells positive for a single contaminant.
- This is an ecological study; the contaminant data do not represent individual exposures.
- The study did not control for other exposures that can impact development, resulting in birth defects.

BOW Study (bow.unl.edu)
A related multidisciplinary project is underway to design and evaluate the feasibility of conducting a case control study in Nebraska. Our overall objective is to assess the risk of adverse fetal outcomes associated with maternal exposure to nitrate and nitrosatable compounds in drinking water. Protocol:
- Identify participants
- 20 cases and 20 controls
- 5 from each water system: Public, private, bottled, filtered
- Questionnaire
- Health history
- Demographics
- Residential/water history
- Saliva sample to measure NO2 and NO3;
- Evaluate maternal nitrosation potential
- Blood sample
- Genetic and chromosomal factors
- Water sample
- Estimate maternal exposure
- Three years prior to conception
- Participant perception
- Motivation and barriers to participate in BOW study

Future work: Fully powered case control study