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EPIDEMIOLOGICAL AND LABORATORY EVIDENCE OF THE EMBRYOTOXIC EFFECTS OF NITRATE AND ATRAZINE INTERACTION

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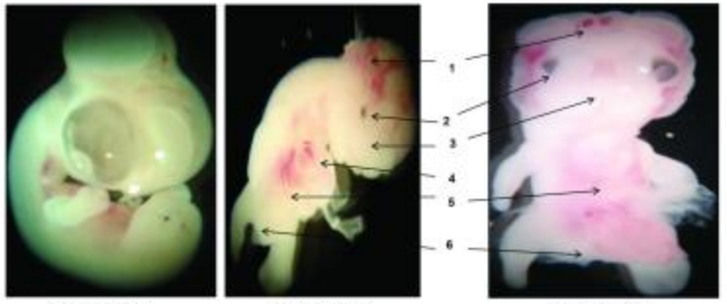
BACKGROUND

Birth defects are a known cause of infant death in the United States[1]. Nitrate and atrazine are widely used agrichemicals found in U.S. drinking water and frequently occur together[2]. While other studies explore the risk of adverse pregnancy outcomes associated with exposure to these agrichemicals as single compounds[3,4], we hypothesize the mixture is more toxic. Nitrate and atrazine can react in vivo at an acidic pH to form *N*-nitrosoatrazine (NNAT), a nitrosamine. Many nitrosamines are known or suspected teratogens.



Normal 5 day | NNAT 0.46µg | NNAT 3.63 µg

1. **Neural tube defect (8%)** - occurs when neural tube fails to close
2. **Microphthalmia (11%)** - abnormally small eye
3. **Craniofacial hypoplasia (11%)** - tissue deficiency or agenesis (failure of organ to develop during embryo development)
4. **Heart defects (24%)** - **Ectopic heart** displacement of heart outside thoracic cavity
5. **Gastroschisis (24%)** - intestines and other organs develop outside abdomen
6. **Caudal regression (19%)** - abnormal development of lower spine

Joshi N, Rhoades MG, Bennett GD, Wells SM, Mirnish SS, Breitbach MJ, Shea PJ Toxicology and Environmental Health, Part A. 2013; 76(17): 1015-1022

EPIDEMIOLOGICAL FINDINGS

Methods

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 Database for Nebraska Groundwater (1977-2014). Contaminants include nitrate and nitrosatable compounds (NCs). Well types include commercial, domestic, irrigation, public water system, monitoring and livestock.

Birth defect rates 2005-2014. Source: Nebraska Department of Health and Human Services
Source for well data: Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater (queried Fall 2015)

Table 1: Association between % + domestic and % + other well types.

Percent Domestic Wells Nitrate Positive	Correlation Coefficient (p-value)
Livestock	0.77 (0.0008)
Public	0.59 (0.0001)
Commercial	0.61 (0.03)
Percent Domestic Wells Atrazine Positive	
Public	0.32 (0.04)
Monitoring	0.55 (<0.0001)
Irrigation	0.45 (<0.0001)

Table 2: Linear regression between birth defect rates and percent of agrichemical positive wells.

Agrichemical (%)*	Slope	p value
Any NCs	3.12	0.02
Only Parent (P) NCs	2.92	0.03
Only Degradate (D) NCs	2.16	0.26
Nitrate	-4.33	0.07
Atrazine	3.03	0.03
Nitrate D	-2.71	0.14
Atrazine D	5.7	0.02
Nitrate P	-6.37	0.02
Atrazine P	1.87	0.05
Nitrate P+D	-2.45	0.19
Atrazine P+D	6.44	0.002
Nitrate+Atrazine D	5.73	0.03
Nitrate+Atrazine P+D	6.9	0.005

*Percent positive of wells tested; D=Domestic wells; P=Public wells; NCs=Nitrosatable compounds

EXPERIMENTAL FINDINGS

Fertilized chicken eggs were acquired from Nelson Poultry Farms, KS and Charles River Laboratories, MA. The experiment was conducted in eight lots of fertilized eggs incubated at 38°C in a humidified, rocking incubator (Little Giant). Each lot consisted of 42 eggs. The analysis was Separated into three different experiments (Table 3).

Outcome: Mortality, weight and embryonic malformations

Exposures: NNAT treatment expressed in µmol/l

Table 3 : Treatment structure

Experiment	Lot	Treatments
1	1, 2, 3	Water Blank DMSO
2	4, 8	DMSO 50:50 Water:DMSO NNAT 0.245 in DMSO
3	5, 6, 7	Blank** DMSO NNAT 1.11 in DMSO NNAT 2.22 in DMSO NNAT 3.33 in DMSO

Table 4: : Effect of NNAT on weight and mortality on eggs from Nelson Poultry farm

Experimental groups	Weight	Mortality
Experiment 1 *Untreated *Water *DMSO	No significant differences **p-value=0.0619**	No significant differences **p-value=0.1141**
Experiment 2 *DMSO *50:50 DMSO/Water *0.245µmol/l	No significant differences **p-value=0.4288**	Could not make conclusions because of zero mortality for two treatments
Experiment 3 *DMSO *1.11µmol/l *2.22µmol/l *3.33µmol/l	No significant differences **p-value=0.1262**	Significant linear increased as NNAT dose increased **p-value=0.0345**

ACKNOWLEDGEMENT

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REFERENCE

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Nitrate-positive domestic wells are correlated to nitrate-positive livestock wells (r=0.77, p=0.0008). Atrazine-positive domestic wells are correlated to atrazine-positive irrigation wells (r=0.45, p=<0.0001) (Table 1)

The largest effect of agrichemicals on birth defect rates is with nitrate + atrazine positive wells (β=6.9, p=0.005) (Table 2)

No significant differences of treatments on weight of the embryo for all three experiments (Table 4).

There was a significant linear increase in mortality as NNAT dose increased (Experiment 3, p=0.0345). These results showed that mortality rates increased with NNAT dose.

The dose response relationship between NNAT treatment and the mortality of the embryos was used to calculate the Lethal dose of NNAT (LD50=2.85 µmol/l)) (Fig 1).

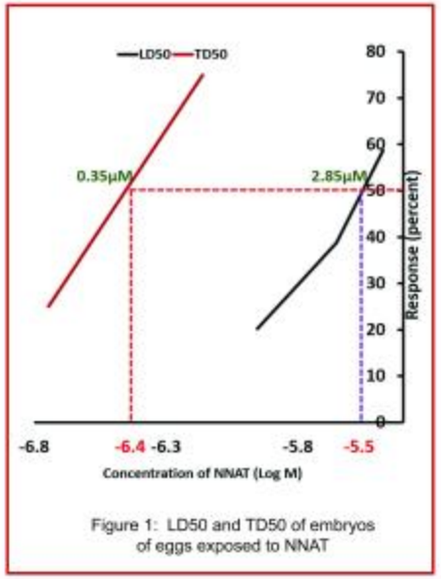

Figure 1: LD50 and TD50 of embryos of eggs exposed to NNAT

Embryos of eggs from Charles River Laboratories developed abnormally after NNAT treatment. The dose that caused embryonic malformation in 50% of Charles River embryos is (TD50=0.355µmol/l) (Fig 1)

CONCLUSIONS

This study suggests that the association between birth defect rates and wells positive for both nitrate and atrazine is stronger than wells positive for a single contaminant in an observational study. In the experimental study we found that NNAT was associated to embryotoxicity and mortality with a TD50=0.355µmol/l and LD50=2.84µmol/l.

Birth Outcomes and Water (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 agrichemicals in drinking water.

Protocol:

- Identify participants: 20 cases and 20 controls
  - 5 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

BIRTH OUTCOMES AND WATER
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