Agrichemical Mixtures in Drinking Water and Birth Outcomes in Nebraska

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Risk Assessment

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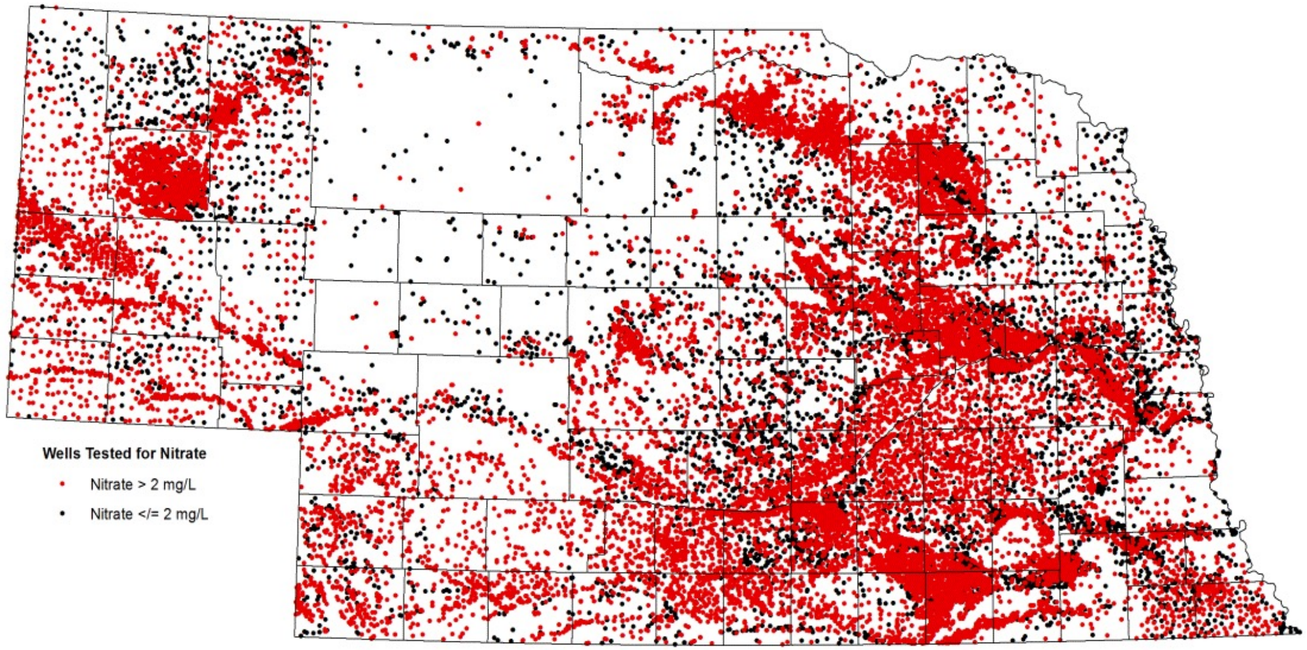
Nitrate and atrazine are the two most prevalent drinking water contaminants in Nebraska.

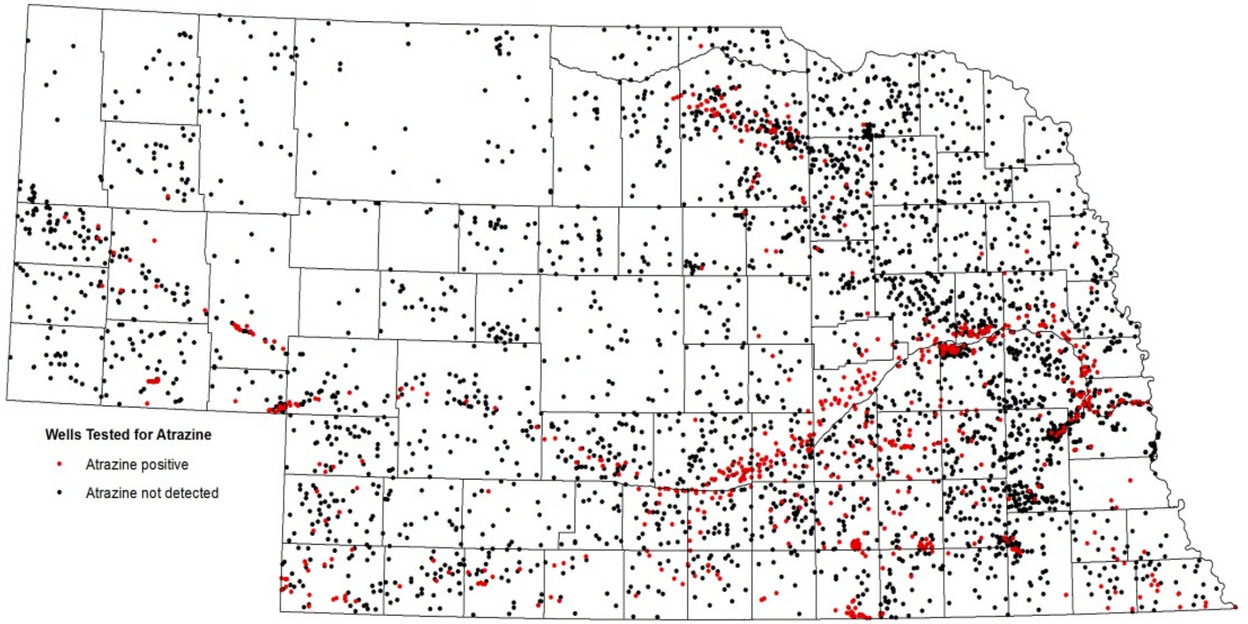
Does exposure increase risk of adverse health outcomes?



Wells sampled for **nitrate** 1977-2014

70% - mean > 2 mg/L

18,513/26,447 wells sampled



Wells sampled for **atrazine** 1977-2014

31% - mean > 0 µg/L

1532 of 4940 wells sampled

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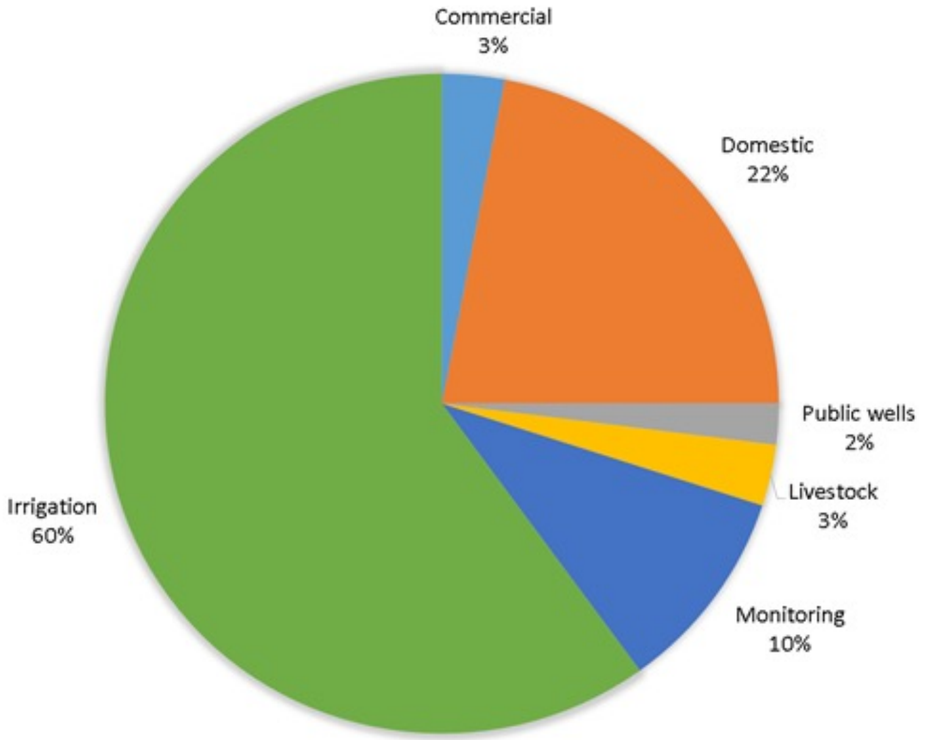
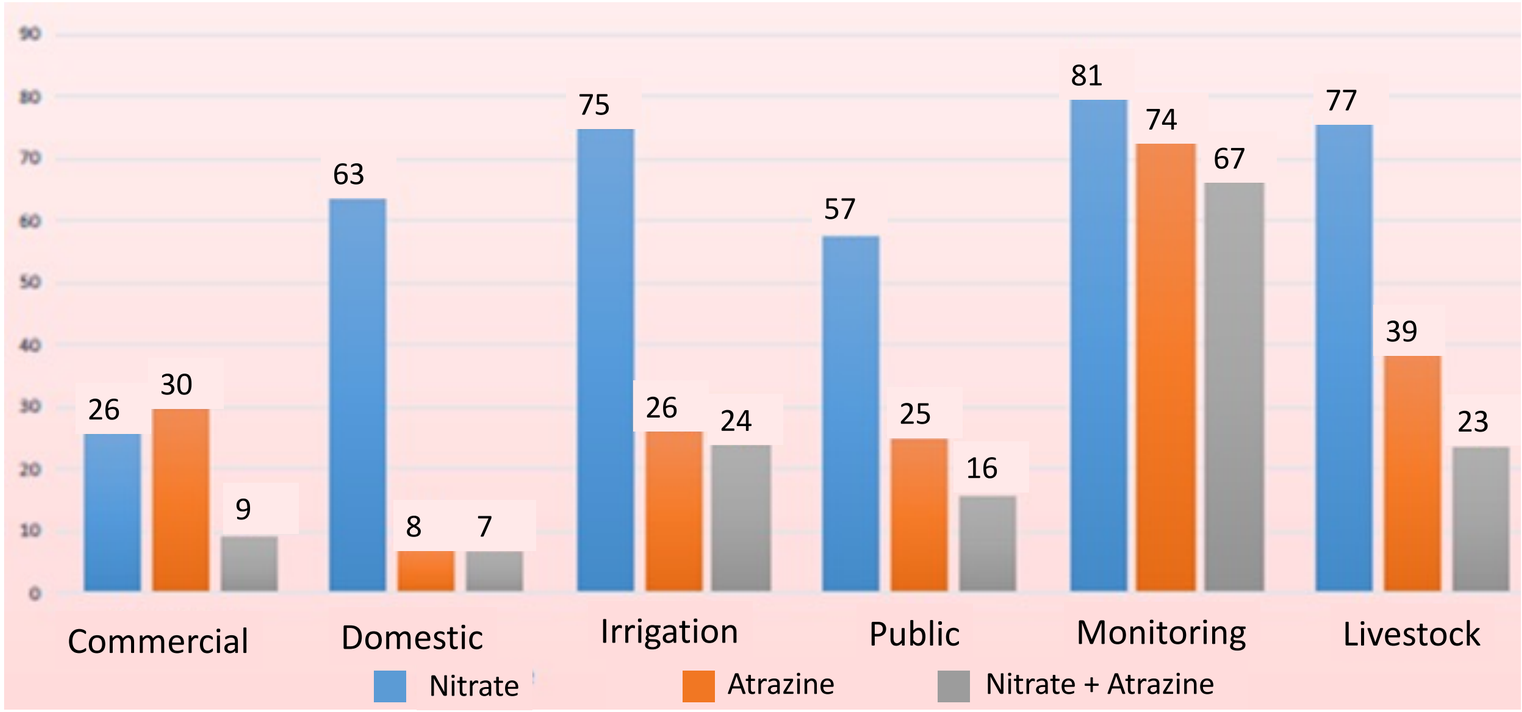
Source: Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater (queried Fall 2015)



Well types sampled (1977-2014)



Percent wells positive for nitrate, atrazine and combination by well type (1977-2014)

Distribution of wells sampled for atrazine and nitrate

Source: Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater (queried Fall 2015)

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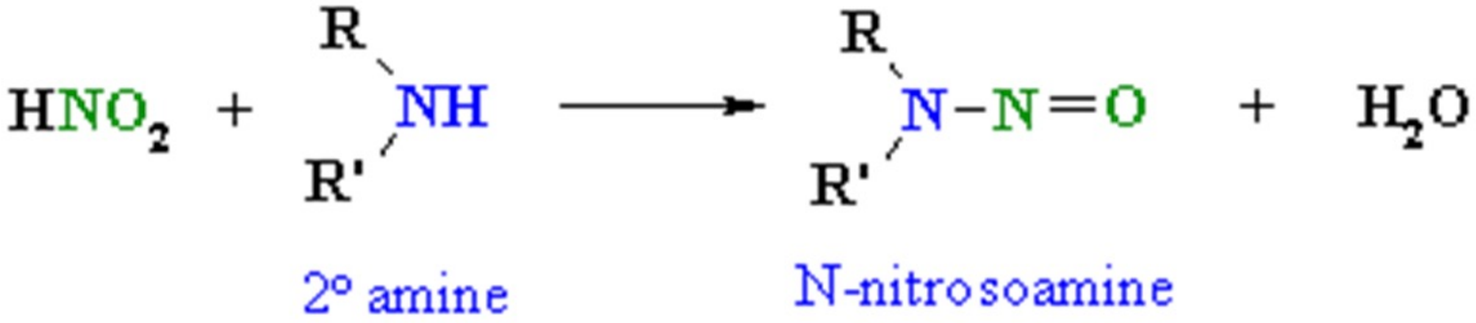
Adverse health outcomes from exposure to nitrate and atrazine in drinking water - is it plausible?



- *N*-nitrosoatrazine (NNAT) easily forms at pH similar to human stomach.
- Many nitrosamines are carcinogenic/teratogenic in animal models.
  - NNAT → chromosomal aberrations in human lymphocytes at doses 1000 X lower than nitrate or atrazine (Meisner, et al. 1993).
  - NNAT→ Malformations in chicken embryos (Joshi, et al. 2013)
  - Risk of NHL increases with exposure to nitrate+atrazine in Nebraska public water systems (Rhoades, et al. 2013)
- **Hypothesis – Exposure to the mixture is more toxic than exposure to either contaminant alone.**

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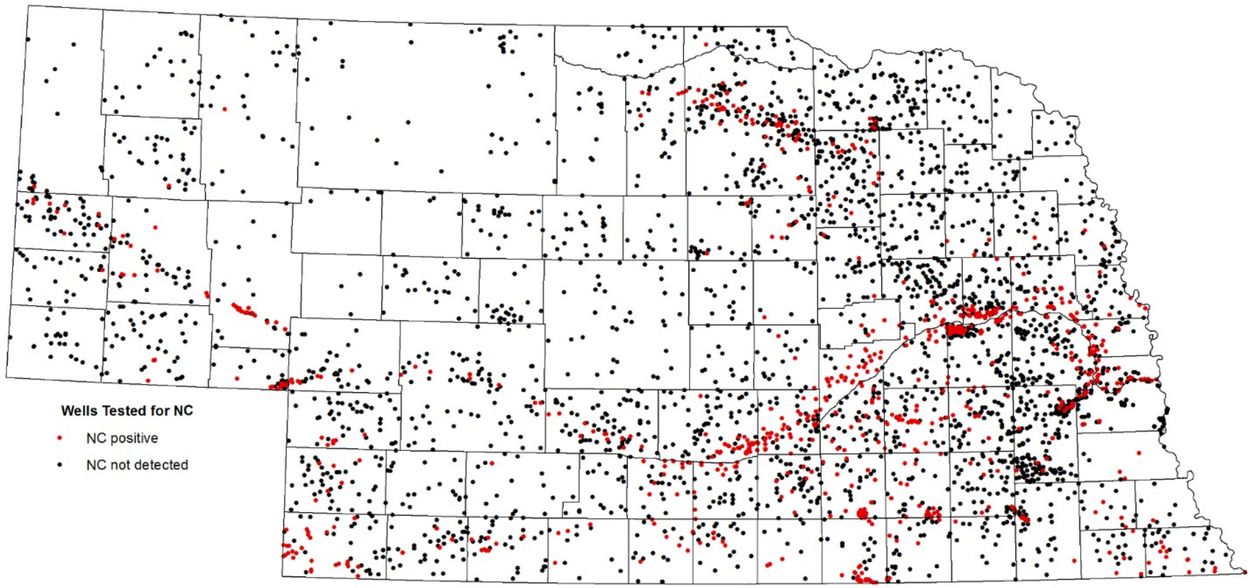
Nitrosatable agrichemicals detected in Nebraska groundwater wells

Metolachlor ESA* 70% (28; 107)	Deethyl-cyanazine 67% (4; 12)	Alachlor ESA* 52% (28; 107)	**Deisopropyl-atrazine 37% (82; 1,927)**	**Deethyl-atrazine 25% (83; 2,081)**	Alachlor ESA* 2°Amide 24% (23; 69)
Propazine 17% (66; 1,988)	Alachlor OA** 16% (19; 56)	Metolachlor OA** 12% (28; 107)	Acetochlor ESA* 11% (28; 107)	Hydroxyalachlor 11% (5; 9)	Hydroxy-simazine 8% (4; 12)
Acetochlor OA** 7% (28; 107)	Alachlor 6% (93; 4,454)	Prometon 4% (87; 2,291)	Acetochlor 3% (77; 1,591)	Bromacil 3% (74; 595)	Simazine 3% (87; 2,430)
Propachlor 2.7% (85; 2,223)	Cyanazine 2% (93; 4,451)	Metolachlor 2% (93; 4,300)	Trifluralin <1% (93; 4,186)	Ametryn <1% (62; 795)	Metribuzin <1% (93; 4,345)
Prometryn <1% (63; 797)	Butylate <1% (93; 4,300)	S-Ethyl-N,N-dipropylthiocarbamate <1% (77; 1,842)	Pendimethalin <1% (75; 1,458)		

Percentage of positive wells tested for nitrate + NC
(# counties; # wells)
1,518 of 4,495 wells sampled were positive for nitrate + NC (34%)

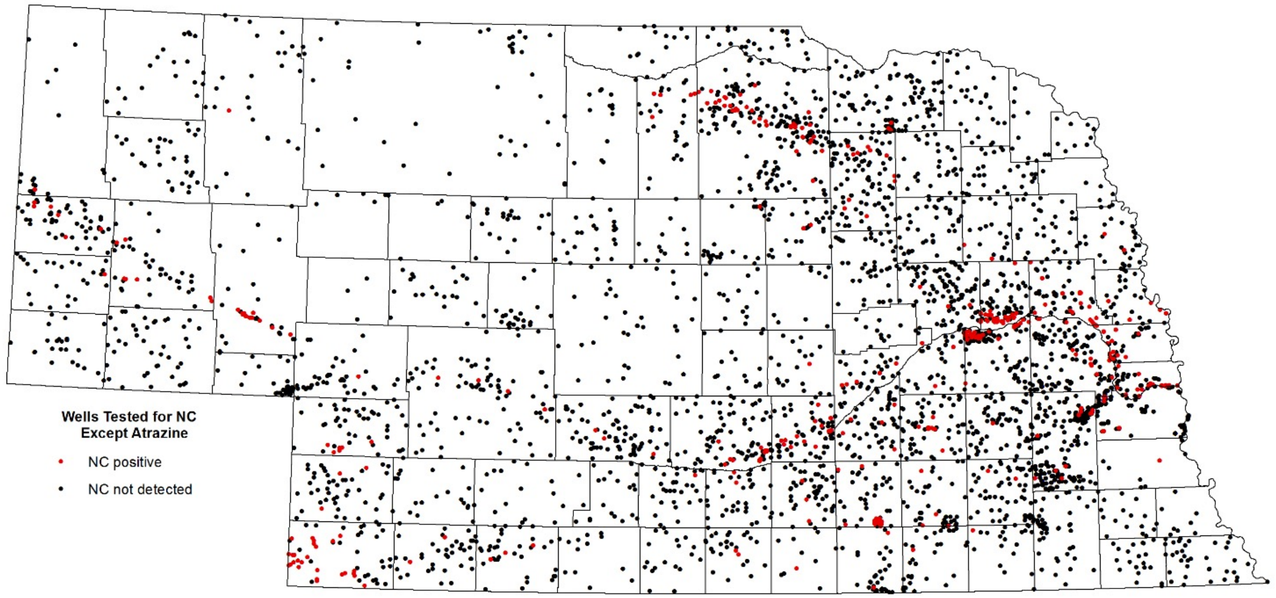
*ethanesulfonic acid
**oxanilic acid

Nitrosatable compounds (NC) detected in Nebraska groundwater wells



Wells sampled for all NC (1977-2014)

24% positive (4736 sampled)



Wells sampled for all NC - atrazine (1977-2014)

18% positive (4736 sampled)

Source: Quality-Assessed Agrichemical Contaminant Database for Nebraska Groundwater (queried Fall 2015)

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Congenital Anomalies in Nebraska

- National rate: Birth defects affect about 3.3% of all live births in the U.S.
- Nebraska rate 2005-2014: 5.8%
- 600-1200 reported birth defect cases per year
    - Cardiovascular (500+)
    - Central nervous system (100+)
    - Gastrointestinal (250+)
    - Genitourinary (550+)
    - Musculoskeletal (250+)

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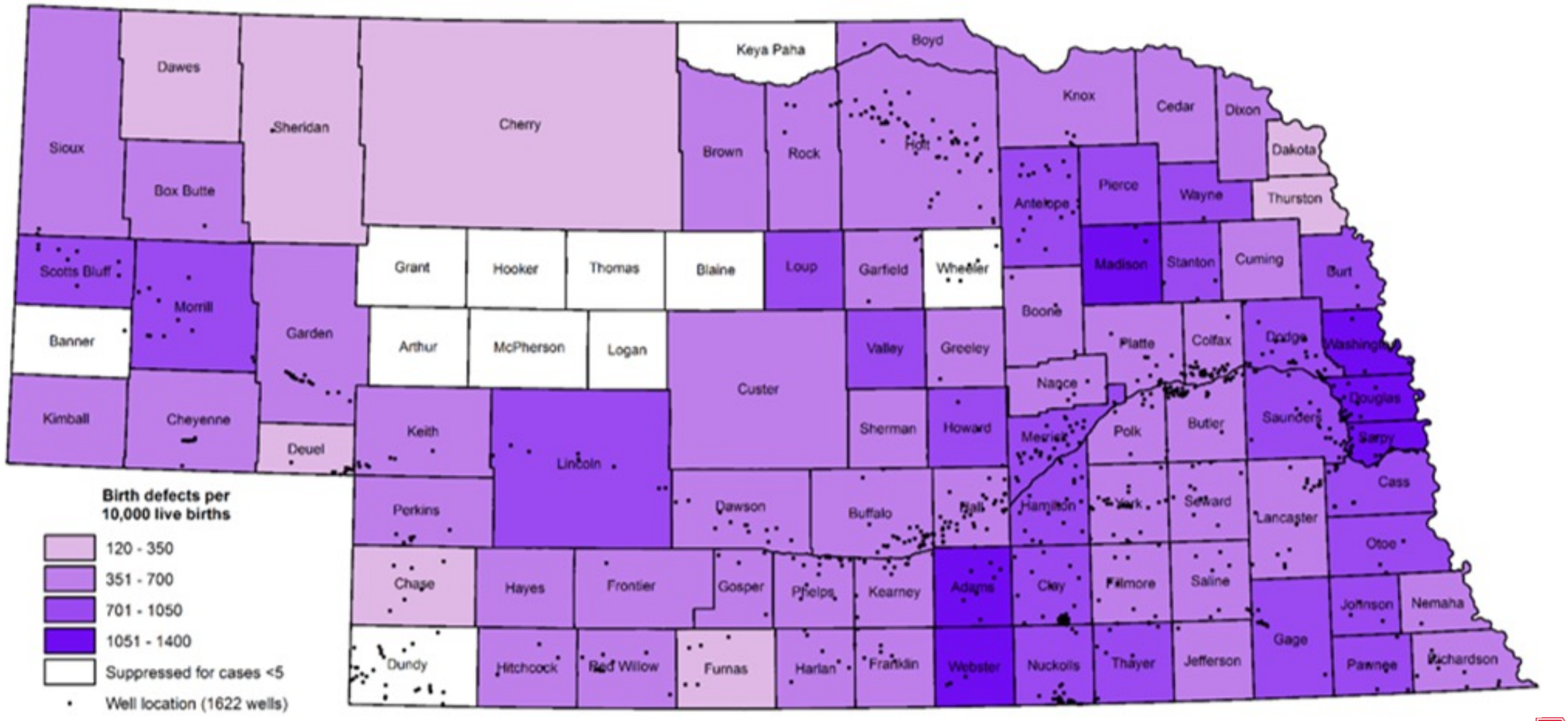
Nebraska birth defect rates by county and wells positive for nitrate + nitrosatable agrichemical



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)

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Evidence from observational study

Linear regression between birth defect rates and percent 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 wells positive
NCs=Nitrosatable compounds
P=Public wells
D=Domestic wells

Wells positive for Nitrate	Estimate	P values
Nitrate (≤2mg)	0	
Nitrate (3-5mg)	-7.4	0.011
Nitrate (≤5mg)	0	
Nitrate (6-10mg)	4.2	0.22
Nitrate (≤10mg)	0	
Nitrate (>10mg)	11	0.0001

Linear regression between birth defect rates and percent nitrate-positive wells

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Correlation between birth defects and percent wells positive by agrichemical

Kendall's tau (τ)	τ	Sig.	No. Counties
All BDs	1.000		93
NO3>0	-.184**	.009	93
NO3+NC	.203**	.005	93
NO3+NC - ATZ	.201**	.007	93
ATZ	.252**	.001	93
NO3 + ATZ	.186*	.011	93
Acetochlor	.223*	.013	77
Acetochlor OXA	.338*	.032	28
NO3+Acet OXA	.338*	.032	28
Alachlor ESA	.472**	.001	28
NO3+Ala ESA	.353*	.012	28
Deethylatrazine (DEA)	.218**	.007	83
NO3+DEA	.213**	.008	83
Deisopropylatrazine (DIA)	.223*	.010	82
NO3+DIA	.222*	.010	82
Metolachlor	.159*	.048	93
EPTC	-.186*	.047	77
NO3+EPTC	-.186*	.047	77

ESA-ethanesulfonic acid
OXA-oxanilic acid

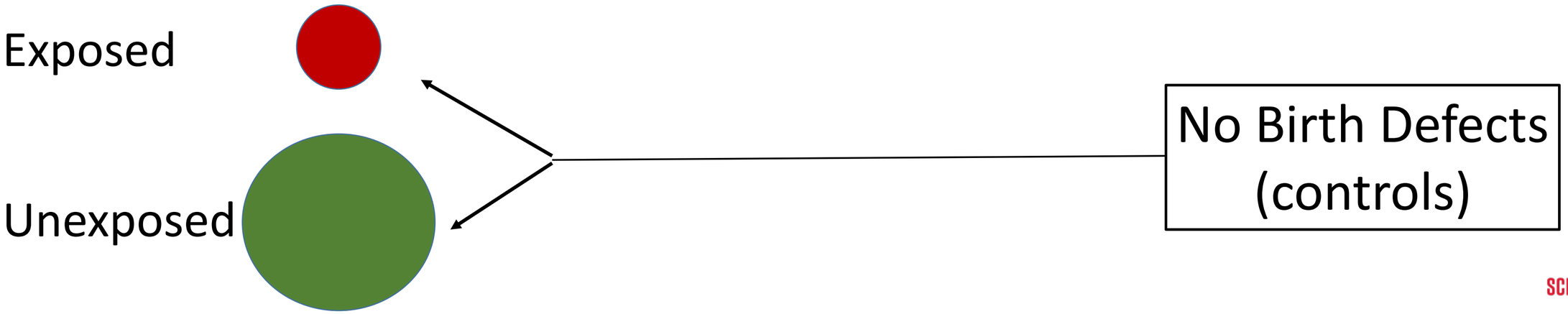
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

ESA-ethanesulfonic acid
OXA-oxanilic acid

Case-Control Studies

The study begins by selecting subjects based on disease status

Look back over time at exposure



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Pilot/feasibility case-control study

bow.unl.edu

- Nebraska women (n=40; 20 cases and 20 controls)
  - 5 each water supply (public, private, bottled, other)
- Questionnaire
  - demographics/health/residential history
- Water sample
  - Nitrate/pesticide analysis
    - Age dating
- Saliva sample
  - Salivary nitrate/nitrite→nitrosation potential
- Blood sample
  - Gene x Environment Interactions
    - Genotyping for *N*-nitrosamine metabolizers (CYP2E1 and NQO1)
    - Chromosomal aberrations – t(14;18)
- Participant Perception
  - Barriers/motivation to participate

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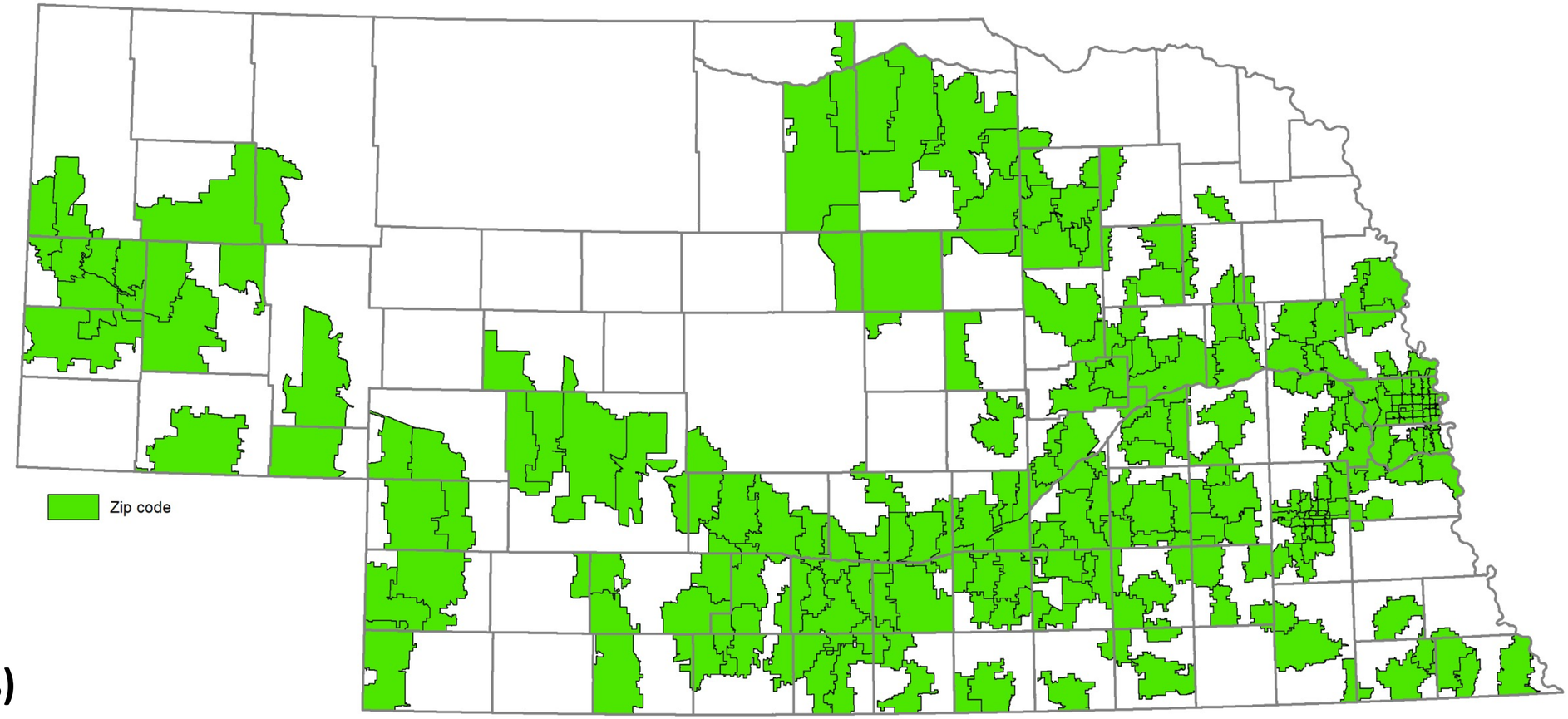
BOW Recruitment

- Zip codes with
  - One well positive for nitrate+nitrosatable agrichemical
  - One birth defect case
  - Lincoln
  - Omaha

(256 zip codes; 41,719 births)



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

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Preliminary Findings

- Two groups of 400
  - 276 undeliverable
  - Left 524
    - Responded: 42
      - Yes: 35
      - No: 7
        - Too busy: 3
        - Not interested: 3
        - Don’t like to participate in research: 1
  - **No response: 482**

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Water source for responders

Type of water	Number of responses
Municipal Water	34
Private Well	4
Rural Water District	0
Bottled Water	8
Other	2

Note: Some responses answered the question of the main drinking water source as two or more categories of the list provided. For example, answered both municipal water and bottled water.
Data source: NDHHS

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Case/control status

	case	control	**total**
Not eligible or lost to followup	8	12	20
Completed	7	1	8
In progress	3	4	7
total	18	17	35

	round 1	round 2
total	19	16

Enrolled more cases than controls

Data source: NDHHS

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Findings to Date

Women are all in or all out.

- Willing to be contacted?
  - Consent to be contacted does not mean consent to participate.

    Yes No
- Willing to be contacted to discuss reasons for participating or not participating?

  Yes No

Participant water source

	Completed	In progress
Public Water System (PWS)	7	2
Private Well	0	2
Bottled	0	1
PWS + Bottled	1	2
TOTAL	**8**	**7**

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Saliva and water data to date

Nitrate/nitrite concentrations

	N	Minimum	Maximum	Mean	Std. Deviation
Nitrate Water (mg/L)	10	.010	2.500	.770	.696
Nitrate Saliva (mg/L)	9	.087	17.277	4.038	7.002
Nitrite Water (mg/L)	10	.000	.124	.016	.038
Nitrite Saliva (mg/L)	9	.045	.997	.385	.323
Valid N (listwise)	9				

Pesticide concentrations

	N	Minimum	Maximum	Mean	Std. Deviation
Atrazine (ug/L)	3	.005	.012	.008	.004
DEA (ug/L)	3	.000	.002	.001	.001
Propazine (ug/L)	3	.000	.005	.002	.003
Valid N (listwise)	3				

Note: 3/3 positive for atrazine, 2/3 positive for DEA, 1/3 positive for propazine

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Participant Perception

- Semi-structured interviews with $25 incentive
- Interviews - 9
  - Water source – public water system
  - Completed all study components - 5
  - Ineligible for BOW study (residential history <3 years) – 1
  - Ineligible for BOW study (3 recruitment contacts w/no response) - 3

Motivators

-Greater good of society

-Interest in study topics (water/birth outcomes)

-Financial incentive

-Importance of research

Barriers

-Time (especially COVID related)

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Next Steps

- Nonresponse letters
  - #2 to Cohort 1
  - #1 to Cohort 2

Limitations

- Residential history
  - Must be a resident at current location for three years prior to conception
    - ? Recruit births 2017-2018?

- Lost to follow-up
  - No response to recruitment call
  - Are these subjects also lost to the participant perception component?

- Well type
  - To date all subjects report public water system as primary drinking water source

- Blood sample
  - Cooperation of providers

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BOW Study Challenges

- Increase awareness of issues and related research
  - Without causing alarm
  - Importance of participation in this type of study
  - Disseminating research findings to the public
- Researcher, community and stakeholder bridge
  - Partnerships
  - Public perception
  - Adapting methodology to increase participation
  - Engagement and collaboration

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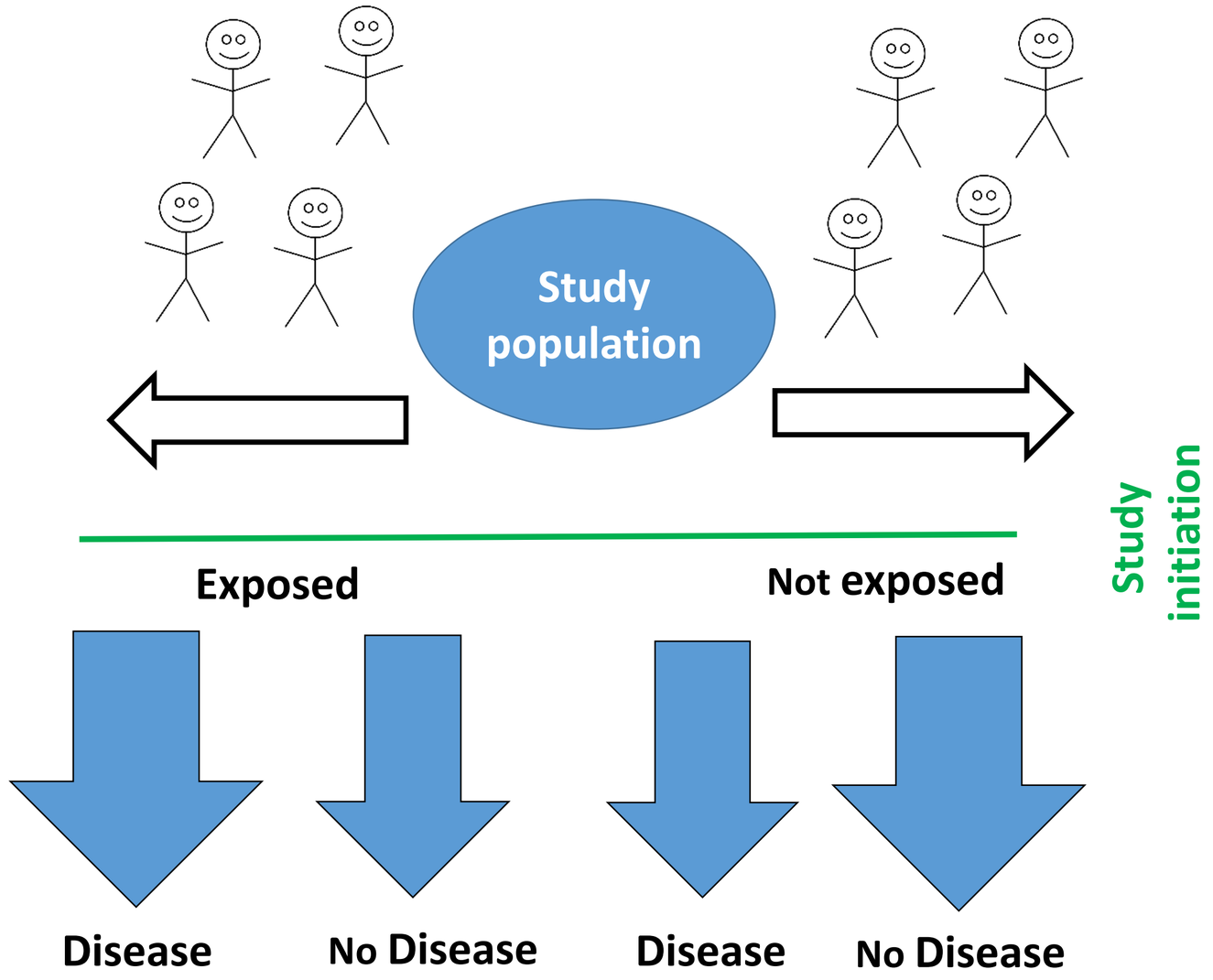
- Nebraska may be a good venue to conduct a cohort study to better understand the human health impacts of agrichemicals in drinking water.
- If there is an association, we can develop preventive measures.



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Thank you!
Questions?