Interactive! Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

TAP TO GO BACK TO KIOSK MENU

Introduction | Methods | Results | Discussion

In 2006, we found that exposure to tap water caused neural tube defects (NTDS) in the rodent colony. NTDs are severe malformations of the brain and spinal cord. The unknown contaminant was present in untreated river water and was not removed during municipal water treatment (Fig 1). No contaminant was identified through regulated water testing. Many naturally occurring elements and pollutants are not regularly monitored in the water system. They are found in low concentrations and are not considered harmful at the levels found. While the contaminants may not be teratogenic on their own, they could interact synergistically. This would make the effect from the combination much greater than the effect from individual components. Thus water contaminants could act together to cause the NTDs observed in mice.

We hypothesized that the NTDs were caused by a synergistic relationship between potentially teratogenic contaminants such as Glyphosate and Atrazine (present in the water at low concentrations), and Zinc, a naturally occurring element. We wanted to explore this relationship further to determine if these combinations could be increasing risk for NTDs.

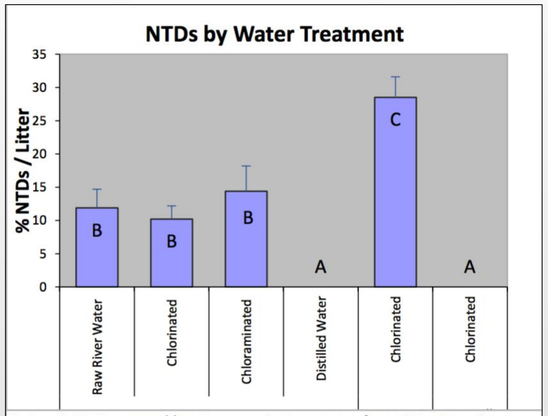

Figure 1. NTDs caused by exposure to tap water from two rivers in southwest Virginia utilizing different water treatment modalities. The water source had a greater effect on NTD formation than the treatment method. The teratogen was present in untreated river water and caused significantly different rates of NTDs between the two river systems.

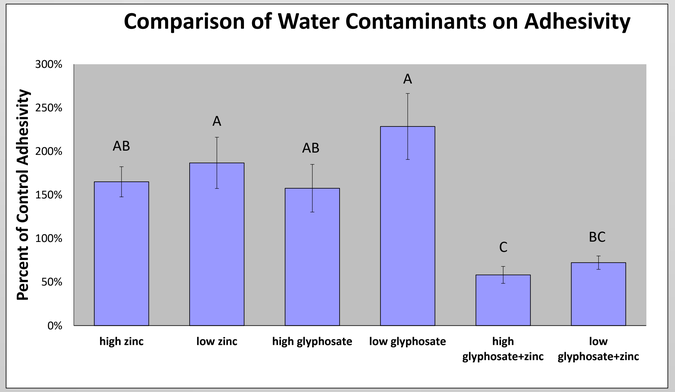

Figure 2. Increased adhesivity is seen in cell culture treated with glyphosate and Zinc , compared to cells treated with just glyphosate or just Zinc.

The C6 glioma Adhesivity Assay is an in vitro assay used to model neural tube defect formation. Increased cell adhesion of rat glial cells to the culture substrate translates to decreased cell migration in the developing neural tube (brain).

We evaluated the viability and adhesivity of the common tap water contaminants Glyphosate and Atrazine, with Zinc, a naturally occurring element found in water sources. Viability was used as a measure of cell toxicity and adhesivity a measure of the likelihood for compounds to produce neural tube defects.

Previous research in our lab found increased cell adhesivity with the glyphosate and naturally occurring Zinc (Zn) at the concentrations found in tap water (Fig. 2) . We also identified that Glyphosate and Zinc acted synergistically. The interaction increased toxicity (measured by cell viability, not shown) and resulted in reduced adhesivity (Fig. 2).

Over 80% of the nation's rivers and streams contain anthropogenic pollutants

For more information on Glyphosate and Atrazine, click here

Interactive!
Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

Glyphosate, the active ingredient in Roundup®, has increased in use with the invent of Roundup Ready® crops that are not adversely affected by glyphosate (Fig. 3). This has allowed farmers to indiscriminately spray fields with glyphosate without worry of harm to their crop. Glyphosate and its metabolite are detected in 36% to 69% of surface water samples and in drinking water (Battaglin et al. 2005).

In animal studies with frogs and chickens, Glyphosate causes craniofacial abnormalities, microcephaly and nervous system defects (Paganelli et al. 2010). Glyphosate has been associated with neurobehavioral birth defects in humans (Garry et al. 2002).

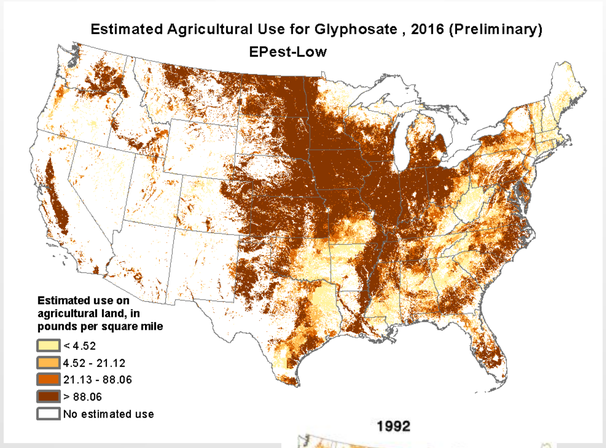

Figure 3. Increase in glyphosate use from 1992 to 2016

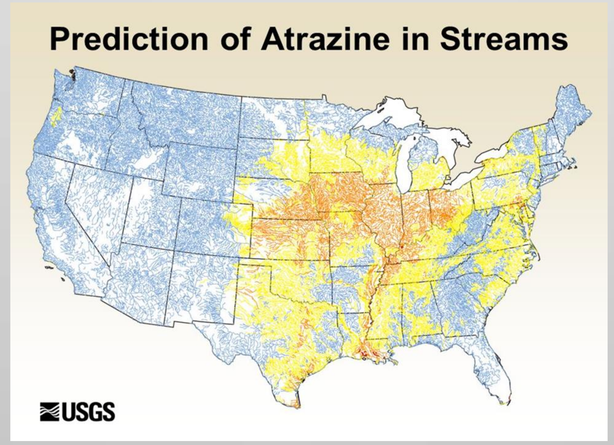

Figure 4. Prediction of Atrazine in streams based on herbicide use and runoff models

Atrazine, another herbicide, is used mainly on corn and sugarcane crops. It is also used for weed control on roadways and golf courses (ATSDR, 2003). Atrazine is a common environmental contaminant, and is often found above the level allowed in surface water (Fig. 4)

Atrazine exposure in humans is linked with small for gestational age babies (Chevrier et al. 2011). Furthermore, a significant association between seasonal elevations in agrichemical use and birth defects in general suggests these compounds may be associated with further defects (Winchester et al. 2009).

Over 80% of the nation's rivers and streams contain anthropogenic pollutants including Glyphosate and Atrazine (Kolpin et al., 2002).

We hypothesized that combinations of anthropogenic pollutants with minerals in the water would cause increased adhesivity, indicating a possible cause for neural tube defects.

Interactive! Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

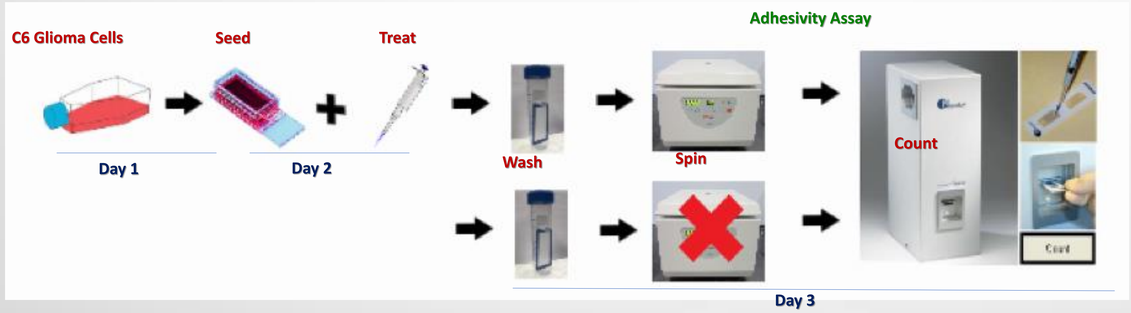

Figure 6. Adhesivity Assay Procedure

Cell Culture:

- C6 Glioma Cells (ATCC CCL-107™) (Fig. 5)
  - Cloned from a rat glial tumor induced by *N*-nitrosomethylurea by Benda et al. after a series of alternate culture and animal passages.

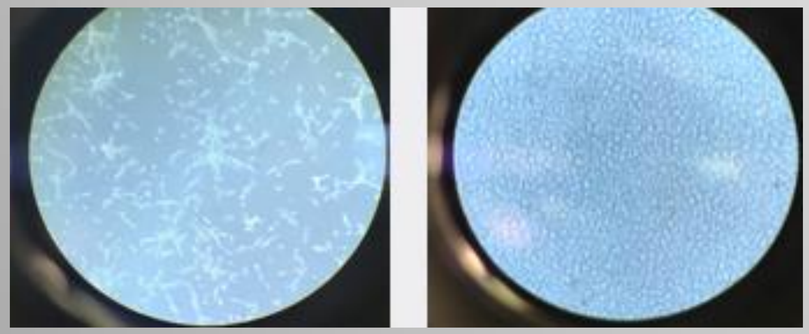

Figure 5. C6 Glioma cells in culture

The C6 glioma adhesivity assay is an in-vitro model of neural tube formation. It is used to screen compounds for the potential to cause NTDs. The neurulation process involves the migration of cells in the neural plate to form the neural folds. Any changes in adhesion and, thus the ability of the cells to migrate to their final destination can cause a NTD (McClay DR, et al., 2001).

To test cell migration on a cellular level, adherence or adhesivity of C6 Glioma cells to glass slides is quantified. Compounds that cause NTDs increase the adhesivity of C6 glioma cells to the slide compared to controls.

Adhesivity Assay (Fig. 6):

- C6 glioma cells were seeded at 30,000 cells/well on a treated glass microscope slide with a removable polystyrene media chamber and grown at 37°C, 5% CO_2 for 24 hours.
- Culture media then replaced with media containing water contaminants and/or elements and incubated for another 24 hours. Three slides per treatment/control were centrifuged in a swinging-bucket rotor at 3,260 g. The other 3 slides per treatment/control were not centrifuged and remained on the lab bench for an equal amount of time.
- Cells remaining on spun and unspun slides were counted using an automated counter.
  - Percent adhesion was expressed as [adhered cells/total cells] x 100 and compared to controls.
- Viability was measured for the treatment and control groups by % Alamar Blue Reduction to indicate how treatment affected cell mortality. Each condition run in triplicate on 96 well plate

Interactive!
Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

Click on graphs for closer look and more information

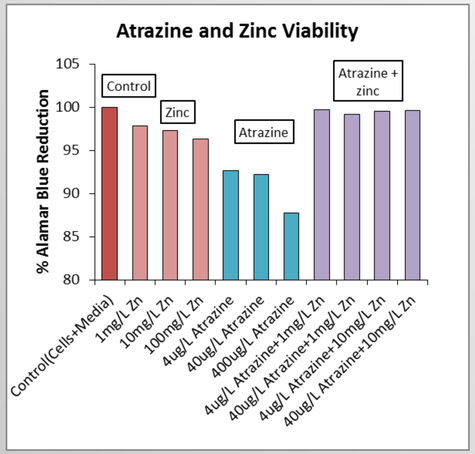

Figure 7. Viability measured by % Alamar Blue Reduction. Zinc showed slight decreased viability with dose response. Atrazine was toxic to cells with clear dose response. Zinc combined with Atrazine had a protective effect, regardless of Atrazine concentration.

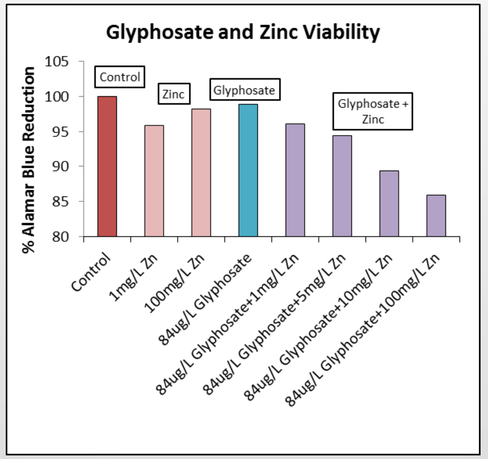

Figure 8. Viability measured by % Alamar Blue Reduction. Glyphosate alone showed no affect on viability. Glyphosate with Zinc showed synergistic effect with a clear dose response with increasing Zinc.

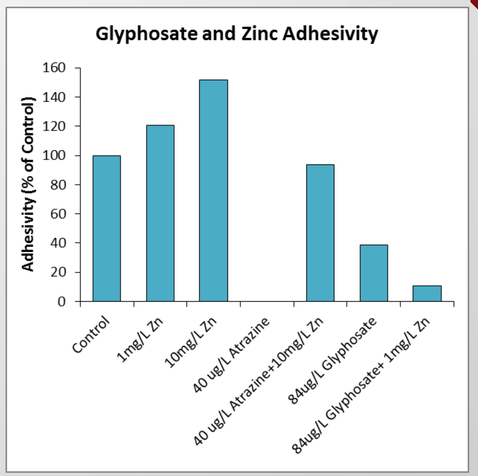

Figure 9. Adhesivity of C6 glioma cells with Glyphosate, Atrazine, and Zinc treatments. Zinc increased adhesivity in a dose dependent manner. Atrazine, at 40 ug/L was toxic to the cells causing them to lift off the slide. Glyphosate alone decreased adhesivity. This effect was enhanced with the addition of Zinc, similar to the combined effect on viability.

Viability is a measure of cell toxicity. Adhesivity assesses the likelihood for compounds to produce neural tube defects. Increased adhesivity means an increased likelihood of NTDs. Viability and Adhesivity need to be evaluated together. Slight changes in cell viability (mild toxicity) can weaken cells such that cells lyse when centrifuged during the adhesivity assay. This was observed with both Atrazine and Glyphosate. Atrazine reduced viability and caused cell lysis during centrifugation; Zinc was protective and restored adhesivity to control levels. Glyphosate did not display decreased viability in unspun slides, but did decrease viability in the spun slides resulting in lower adhesivity scores. Lysis of cells on the spun slide was enhanced with the addition of Zinc. Additional concentrations of glyphosate, which do not affect the viability of spun cells, need to be tested in order to determine the actual effect on cell adhesivity and thus ability to cause NTDs.

Interactive!
Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

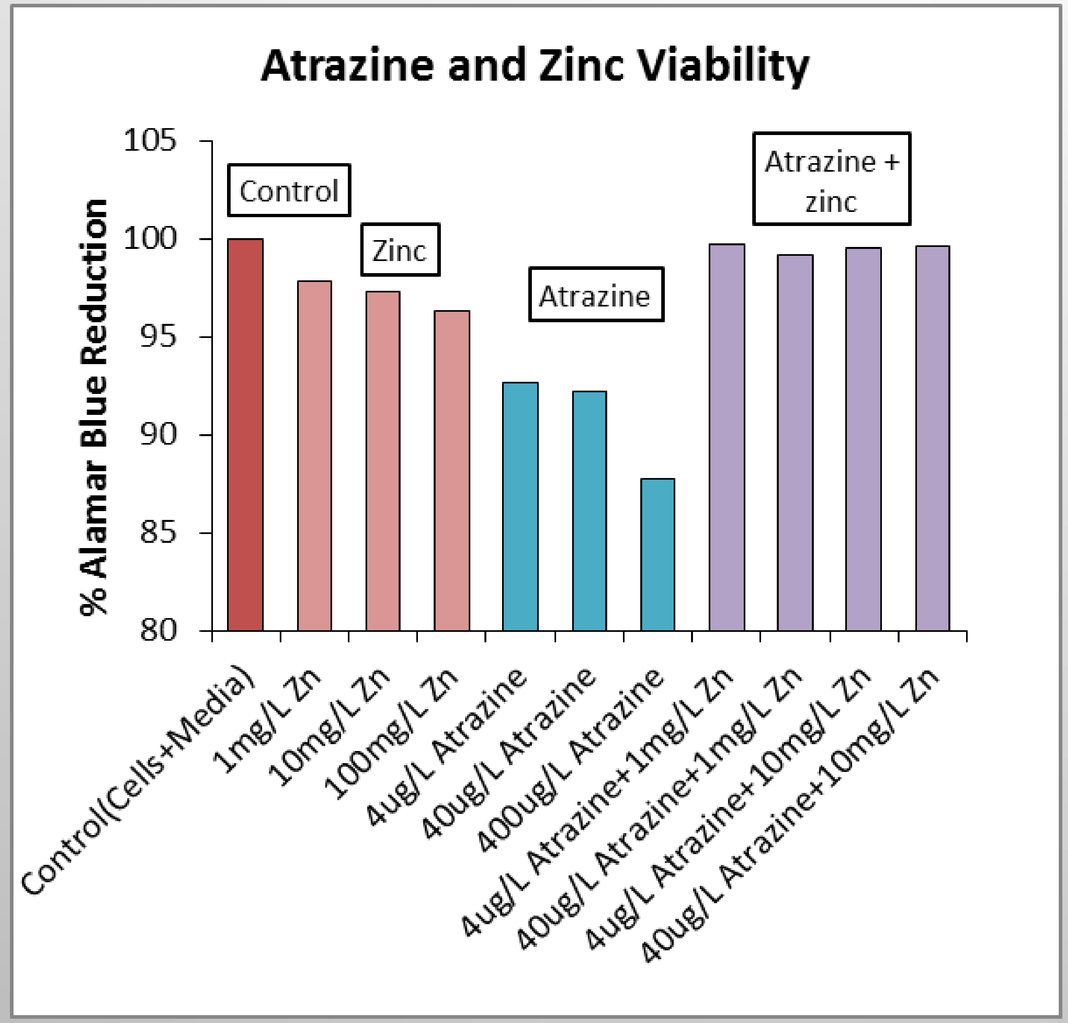

Figure 7. Viability measured by % Alamar Blue Reduction.

Zinc showed slight decreased viability with small dose response.

Atrazine was toxic to cells with clear dose response.

Zinc combined with Atrazine had a protective effect, regardless of Atrazine concentration.

Interactive!
Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

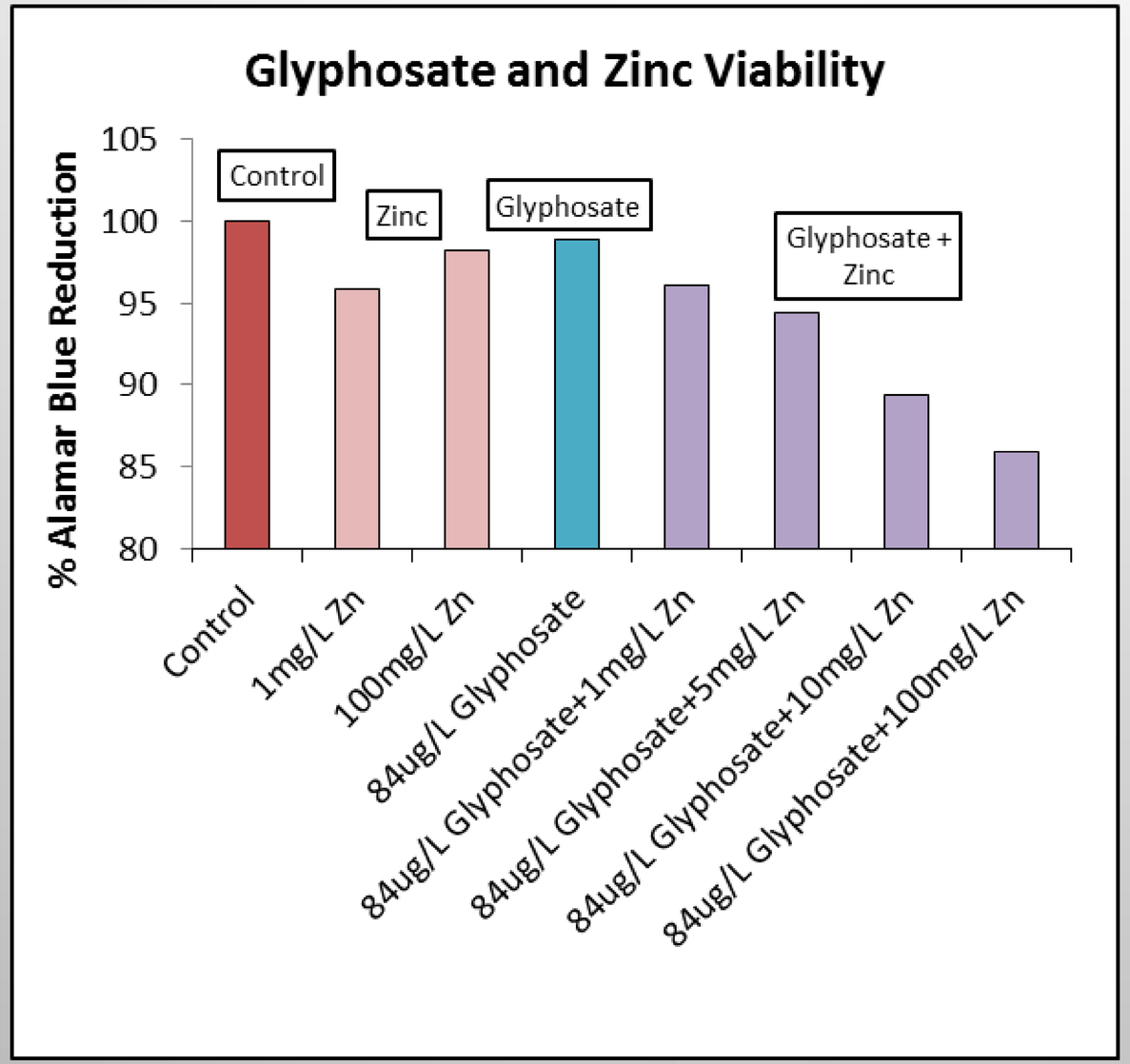

Figure 8. Viability measured by % Alamar Blue Reduction.

Glyphosate alone showed no affect on viability.

Glyphosate with Zinc showed synergistic effect with a clear dose response with increasing Zinc.

Interactive!
Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

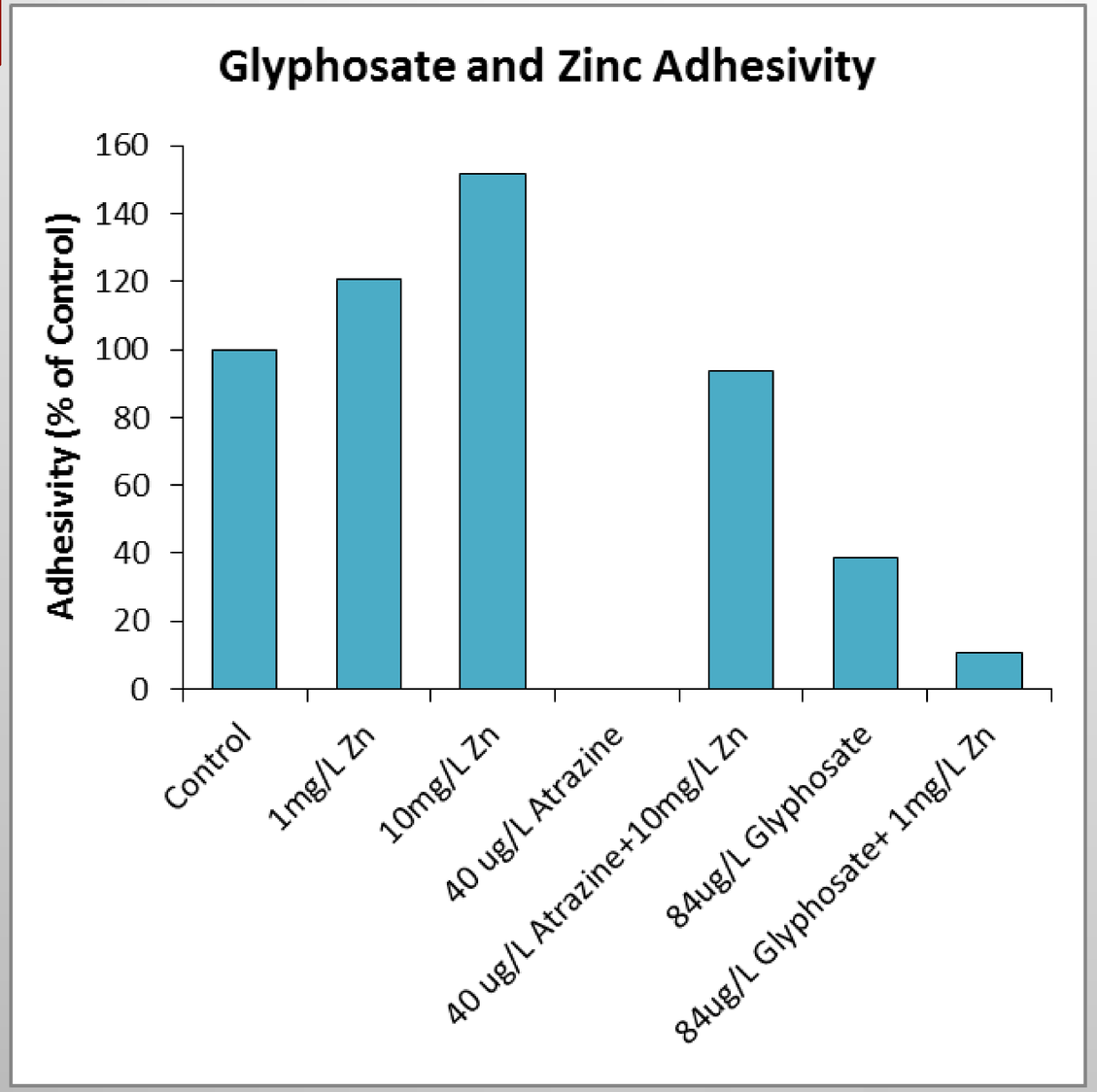

Figure 9. Adhesivity of C6 glioma cells with Glyphosate, Atrazine, and Zinc treatments.

Zinc increased adhesivity in a dose dependent manner.

Atrazine, at 40 ug/L was toxic to the cells causing them to lift off the slide.

Glyphosate alone decreased adhesivity. This effect was enhanced with the addition of Zinc, similar to the combined effect on viability.

Interactive!
Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

The decreased viability when glyphosate and Zinc are combined suggests a synergistic relationship which enhances toxicity. Atrazine was toxic to cells alone; however, in combination with Zinc, toxicity was reduced in both the viability and adhesivity assays. Thus Zinc acts synergistically with atrazine in a protective manner. It is not surprising that Zinc enhances toxicity with one contaminant and decreases toxicity with the other. Each individual contaminant can exert toxicity through separate mechanisms. These mechanisms can respond to the presence of Zinc differently to enhance or reduce toxicity.

These data have important implications for future research and health care concerns. Glyphosate and Atrazine are common contaminants in water and are not removed during water treatment. (Kolpin et al., 2002). Our data imply that glyphosate and Zinc may be involved in NTD formation. This correlates with studies showing a rise in the incidence of birth defects during times with increased agrichemical use (Winchester et al. 2009).

In future research, we would like to explore lower concentrations of glyphosate as well as other combinations of agrichemicals and trace minerals that are present in water. As we find combinations that indicate possible teratogenicity, we will move forward to animal trials.

Additionally, epidemiological studies to test water contaminants identified by this research, and the rates of birth defects in the surrounding population are needed to evaluate the effect on human health.

Click here to view Literature Citied

Interactive! Click on any of these bubbles *to jump* to each section

Tap Water Components and Their Effect on Cell Adhesivity, a Model for Brain Development

Rachel Hall[1], Razan Alajoleen[1], Sara Abdelnour[1], Anam Jafri[1], Chevon Thorpe[2], Terry C. Hrubec[1,3]

1. Edward Via College of Osteopathic Medicine, Virginia Campus, Blacksburg, VA. 2. COS, Virginia Tech, Blacksburg, VA.
3. VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, VA

Literature Cited

Agency for Toxic Substances and Disease Registry (ATSDR). 2003. Toxicological Profile for Atrazine. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM, Sandstrom MW, 2005. Glyphosate, Other Herbicides, and Transformation Products in Midwestern Streams. Journal of the American Water Resources Association 41(2):323- 332.

Borggaard OK, Gimsing AL, 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review, Pest Manag Sci 64:441–456.

Chevrier C, Limon G, Monfort C, Rouget F, Garlantezec R, Petit C, Durand G Cordier S, 2011. Urinary biomarkers of prenatal atrazine exposure and adverse birth outcomes in the PELAGIE Birth Cohort. Environmental Health Perspectives, 119:1034-41.

Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, Burroughs BL, 2002. Birth Defects, Season of Conception, and Sex of Children Born to Pesticide Applicators Living in the Red River Valley of Minnesota, USA Environmental Health Perspectives 110:S3.

Heberer T, et al., 2002. From municipal sewage to drinking water: fate and removal of pharmaceutical residues in the aquatic environment in urban areas, Water Sci Technol, 46:81-8.

Mallela MK, Were SD, Hrubec TC, 2010. Neural tube defects in mice exposed to tap water. Environmental Toxicology. 2012, 26(6):633-640.

McClay DR, et al., 2001. Quantitative Measurement of Cell Adhesion Using Centrifugal Force. Current Protocols in Cell Biology. 9.2.1–9.2.10.

Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE, 2010. Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling. Chem. Res. Toxicol., 2010, 23 (10), pp 1586–1595.

Stackelberg PE, et al., 2007. Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. Science of the Total Environment 377:255-272.

VA DEQ, Virginia Department of Environment Quality, 2016. Reduction of Toxics in State Waters Report - January 2015. http://www.deq.virginia.gov/Programs/Water/WaterQualityInformationTMDLs/ WaterQualityMonitoring/ToxicsReport-January2015.aspx

Winchester PW, Huskins J, Ying J, 2009. Agrichemicals in surface water and birth defects in the United States. Acta Pædiatrica 98:664–669.