Published in final edited form as:

Environ Toxicol. 2011 November ; 26(6): 633–640. doi:10.1002/tox.20600.

Neural Tube Defects In Mice Exposed To Tap Water

Murali K Mallela¹, Stephen R Werre², and Terry C Hrubec^{1,3}

Murali K Mallela: mallela@vt.edu; Stephen R Werre: swerre@vt.edu

¹ Department of Biomedical Sciences and Pathobiology, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061

² Laboratory for Study Design and Statistical Service, VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061

³ Department of Biomedical Sciences, E. Via Virginia College of Osteopathic Medicine, Blacksburg, VA 24060

Abstract

In May of 2006 we suddenly began to observe neural tube defects (NTDs) in embryos of untreated control mice. We hypothesized the mice were being exposed unknowingly to a teratogenic agent and investigated the cause. Our results suggested that NTDs were not resulting from bedding material, feed, strain or source of the mice. Additionally, mice were negative for routine and comprehensive screens of pathogens. To further test whether the NTDs resulted from infectious or genetic cause localized to our facility, we obtained three strains of timed pregnant mice from commercial suppliers located in 4 different states. All strains and sources of mice arrived in our laboratory with NTDs, implying that commercially available mice were possibly exposed to a teratogen prior to purchase. Our investigation eventually concluded that exposure to tap water was causing the NTDs. The incidence of NTDs was greatest in purchased mice provided tap water and lowest in purchased mice provided distilled deionized water (DDI). Providing mice DDI water for two generations (F2-DDI) eliminated the NTDs. When F2-DDI mice were provided tap water from three different urban areas prior to breeding, their offspring again developed NTDs. Increased length of exposure to tap water significantly increased the incidence of NTDs. These results indicate that a contaminant in municipal tap water is likely causing NTDs in mice. The unknown teratogen appears to have a wide geographic distribution but has not yet been identified. Water analysis is currently underway to identify candidate contaminants that might be responsible for the malformations.

Introduction

Neural tube defects (NTDs) are serious birth defects where the malformed brain or spinal cord is uncovered by bone or skin, resulting in anencephaly and spina bifida. NTDs are the second most common malformation after congenital heart defects (Christianson et al., 2006). Many if not most birth defects have a multifactorial etiology where embryonic development of a genetically susceptible fetus is altered by environmental factors. Several environmental, genetic and nutritional factors have been shown to cause NTDs in mice including drugs, physical agents (hyperthermia), vitamin excess or deficiency, maternal infectious and metabolic diseases. In mice, differential neural fold elevation initiates neural tube closure at multiple sites. Failure of neural fold closure at these sites leads to variety of malformations such as exencephaly, spina bifida, split face, or rachischisis depending on location of the

open neural tube (Harris and Juriloff, 1999). Historically, the CD-1 strain of mouse has a low background rate of 1/500 to 1/1000 NTDs (Dr. C. Clifford, Charles River Labs; personal communication). Consistent with this low rate of spontaneous NTDs, our lab has examined over 1700 fetuses (>130 litters) prior to May, 2006 without encountering a single NTD. Beginning in May of 2006, we began to observe NTDs in embryos of untreated CD-1 mice. As NTDs appeared suddenly and have persisted since that time, we investigated the cause of this surprising finding. Studies were conducted to examine feed, water, housing location, bedding material, mouse strain and exposure to infectious agents as possible causes for the NTDs in control mice.

Materials and Methods

Animals

Three sources of mice were used in these experiments: 1) Purchased CD-1 mice were obtained from Charles River Labs at 5–8 weeks of age and housed in conventional cages in a standard mouse room with 12 hours day length, 70–73°F, 50±20 % humidity and *ad libitum* access to feed and water. 2) Time pregnant mice (CD-1 strain from Company A, Raleigh NC and Portage MI; ICR strain from both Company B, Indianapolis, IN and Company C, Germantown, NY, and Swiss Webster mice from Company C, Germantown, NY) were bred at the vendor's facility and shipped on gestation day (GD) 10–11 after neural tube closure. Embryos were evaluated for NTDs immediately upon arrival in our facility on GD 11–12. 3) F2-DDI mice were produced in our facility by breeding CD-1 mice provided only DDI water for two generations (Figure 1).

For breeding, 2 or 3 females were added to a male provided with the same experimental conditions. The females were checked for copulation plugs every morning. When a copulation plug was found, the female was separated, and midpoint of the preceding dark cycle was designated as gestation day (GD) 0. Mice were euthanized on GD 9.5 by an overdose of CO₂ and embryos were evaluated for neural tube defects under a stereozoom microscope (Olympus SZX7, Melville, NY). Embryos were staged by somite count, number of branchial arches and amount of limb bud growth to verify sufficient development for neural tube closure.

The feed was certified by the manufacturer (Teklad diets, Madison, WI) using independent analysis for potential contaminants. Bedding material was tested independently by the manufacturer (ALPHA-Dri, Shepherd Specialty Papers, Watertown, TN) for possible contaminants including arsenic, bismuth, cadmium, lead, organophosphate pesticides, organochlorine pesticides, PCBs. Experimental and sentinel mice were tested numerous times over a 7 month period for routine and comprehensive screens of known mouse pathogens (Rodent & Rabbit Serology Laboratory at BioReliance, Rockville, MD).

Experimental Conditions to Screen for Possible Risk Factors

Purchased CD-1 mice were exposed to multiple combinations of housing, diet and water for 2 weeks in order to narrow down possible causes for the observed malformations. To test whether the location or type of housing affected NTD rates, newly purchased CD-1 mice were maintained in conventional cages (n= 92), in microisolator cages under sterile conditions in a HEPA filtered laminar flow hood (n=9), and under Biosafety Level 2 (BSL2) containment conditions in a separate facility (n=3). Four different commercial rodent diets including Harlan Teklad Rodent Diet 7013 (n=3), Madison, WI; Ziegler Rodent diets 18-6 (n=61) and NTP-2000 (n=9), Gardeners, PA and Purina 5001 (n=31), Richmond, IN, USA were tested. All are complete diets suitable for growth and reproduction. Three different sources of water were tested in the purchased CD-1 mice including tap water from Location

A, south west VA (n=25), distilled-deionized (DDI) water (Milli-Q system water at 18 Ohm resistance, Millipore Corporation, Billerica, MA) (n=30), and HPLC water (OmniSolv, EMD Chemicals Inc, Gibbstown, NJ) (n=26). Mice received fresh water once or twice a week. Mice that were maintained under sterile conditions received new autoclaved water once or twice a week. Water was provided in either glass or plastic water bottles. For the initial studies, water was provided in plastic water bottles, all subsequent studies were conducted using glass water bottles; there is no association of NTDs with the type of bottle used. All water was periodically tested for total and free chlorine, ammonia, nitrate, hardness, alkalinity, and pH. Electrical resistance was monitored continuously for DDI water system.

In our last experiment, F2-DDI mice were tested with 5 sources of water under controlled conditions. All mice were housed in conventional cages and were fed Zeigler NTP diet. The 5 sources of water were: municipal tap water from Location 1 in southwest Virginia (n=25); municipal tap water from Location 2 in northeast MD (n=23); municipal tap water from Location 3 in central MD (n=21); DDI water (n=20); and HPLC water (n=8). Among these, Location 2 and 3 are from urban commercial water suppliers while location 1 is from a rural commercial water supplier. Fresh water from the 5 sources was provided to the mice once or twice a week.

All procedures related to animal use were approved by and conducted in compliance with the Institutional Animal Care and Use Committee (IACUC) at the College of Veterinary Medicine at Virginia Tech, an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility.

Statistical Analysis

Statistical analysis was conducted by the Laboratory for Study Design and Statistical Service at Virginia Tech. Dams were considered the treatment unit and the incidence of NTDs were calculated as the mean percentage of embryos with NTDs per litter. Associations between treatment variables and incidence of NTDs were assessed by Poisson regression using the Statistical Analysis System (version 9.1.3, SAS Institute Inc., Cary, NC 27513) and a statistical significance was set at $p \leq 0.05$.

Results

In our study, the embryos showed failure of neural fold closure at 3 distinct locations resulting in spina bifida, exencephaly, split face defect (Figure 2).

Screening for Infectious and Toxic Exposure

The feed analysis demonstrated the feed to be free of heavy metals, organophosphates, and chlorinated hydrocarbons. Several epidemiological and animal studies have shown that fungal toxins such as fumonisin can induce neural tube defects (reviewed by Cabrera et al., 2004; Marasas et al., 2004); however, the feed when analyzed, tested negative for aflatoxin and fumonisin, two common contaminants in corn based feeds. Bedding material was free of arsenic, bismuth, cadmium, lead, organophosphate pesticides, organochlorine pesticides, PCBs and other contaminants. The mice were also consistently negative on all of the serologic tests conducted (data not shown).

Association of Housing and NTDs

Purchased mice held under all three housing conditions continued to produce embryos with NTDs (Figure 3).

NTDs in Timed Pregnant Mice from Commercial Suppliers

To further test whether the NTDs resulted from unknown infectious or genetic causes localized to our facility, timed pregnant mice of different strains were shipped from suppliers on GD 10–11 after the time of neural tube closure. Embryos of timed pregnant mice were examined for NTDs within 1 hour upon arrival at our facility. Embryos from all 3 strains and from all suppliers exhibited NTDs (Figure 4) with no significance differences ($p=0.1271$) between strain or supplier.

Association of Diet and NTDs

The incidence of NTD formation was compared in fetuses of newly purchased dams provided 4 different commercial rodent diets for 2 weeks prior to breeding. There was no significant effect of diet on the incidence of NTDs ($p=0.7024$) (Figure 5).

Association of Source of Water and NTDs

NTD rates were compared in embryos from newly purchased mice provided 3 different sources of water for 2 weeks prior to breeding: tap water (location 1), DDI water and HPLC water. Embryos from mice provided DDI water ($p=0.0046$) or HPLC water ($p=0.0011$) had significantly lower incidence of NTDs compared to tap water (Figure 6).

F2 Generation: Association of Water and NTDs

Mice were bred for two generations and provided only DDI water, to ensure no exposure to the teratogen. When these F2-DDI mice were bred, NTDs were completely eliminated in the offspring (Figure 7).

NTDs were not observed when F2-DDI mice were transferred back to HPLC water for 2 weeks prior to breeding. When F2-DDI mice were provided tap water from Location 1, Location 2, or Location 3, for 2 weeks prior to breeding, NTDs were observed in all groups of tap water exposed mice (Figure 7). The comparisons, however, did not reach statistical significance compared to DDI water (p -values: 0.1895, 0.1304, 0.0581 for Locations 1, 2, 3 respectively). When the exposure length was increased to 8 weeks prior to breeding, all three groups of mice had significantly higher rates of NTDs than mice exposed to DDI water (p -values: 0.0032, 0.0050, 0.0073 for Locations 1, 2, 3 respectively). Longer periods of exposure to tap water from all municipalities showed a dose response effect. The same relative incidence of NTDs between the three locations was maintained at both exposure times.

Discussion

Approximately 292 million people in the United States rely on community water systems for their drinking water needs (U.S EPA, 2008). Most municipal water comes from surface water which is increasingly contaminated with agricultural or man made pollutants. Contamination of surface water with pathogens and toxins significantly impacts human and animal health. The US Geological Survey found in 2002 that 80% of the nation's rivers and streams were contaminated with wastewater contaminants. The most frequently detected compounds in surface water were cholesterol, coprostanol (fecal steroid), *N*, *N*-diethyltoluamide (DEET - insect repellent), caffeine, triclosan (antimicrobial disinfectant), tri(2-chloroethyl)phosphate (fire retardant), and 4-nonylphenol (sewage treatment surfactant, and endocrine disruptor) (Kolpin et al., 2002). Other ubiquitous contaminants of drinking water include trihalomethanes, haloacetic acid, MTBE, phthalates, synthetic estrogens and other pharmaceuticals, atrazine, DDT, benzene, toluene, xylene, tributyl tin, arsenic, fluoride, selenium, and heavy metals (Schwarzenbach et al., 2006). The Safe Drinking Water Act sets the limits for the maximum allowable concentrations of water contaminants.

Safe concentrations in municipal drinking water have not been established for some of these contaminants, nor is their presence monitored (Schwarzenbach et al., 2006). Specific contaminants may not be present in toxic concentration in drinking water, but mixtures can act synergistically such that the resulting combination is toxic. Little is known about the potential toxicity or long term human health effects from chronic exposure to mixtures of these common water contaminants. Contamination of drinking water sources with agricultural herbicides adversely affects reproductive health including intrauterine growth retardation, low birth weight and prematurity (Ochoa-Acuña et al., 2009, Villanueva et al., 2005, Munger et al., 1997).

Recent studies have found trace amounts of pharmaceuticals and endocrine disruptors in both surface and drinking water, although, adverse health effects from this contamination have not been positively identified (Reviewed by Snyder and Benotti, 2010). Pharmaceuticals and their metabolites can enter the environment or waste water from excretion of those compounds, or from disposal of expired and unused pharmaceuticals. Common pharmaceutical compounds such as antibiotics, anti-inflammatory agents, anti-epileptics, antacids, diuretics and β blockers have been found in a variety of water sources including surface and ground water (Fent et al., 2006; Glassmeyer et al., 2008 reviews). In another study conducted from 19 U.S. water utilities, the most frequently detected contaminants were atrazine, carbamazepine, estrone, gemfibrozil, meprobamate, naproxen, phenytoin, sulfamethoxazole, TCEP and trimethoprim (Benotti et al., 2008). The stable chemical structure of some organic contaminants, including various pharmaceuticals and endocrine disruptors, may prevent their removal by water treatment systems (Stackelberg et al., 2004, 2007) (Falconer, 2006). Occurrence of contaminants in finished drinking water may also depend on type of water treatment (Benotti et al., 2008).

Disinfection of water is necessary to prevent water-borne diseases such as cholera, typhoid and cryptosporidiosis. Common disinfection methods use chemical oxidation by chlorine, chloramines, ozone or chlorine dioxide depending on the water quality and supply system. The disinfectants can react with other compounds present in the water producing toxic by-products (Rice et al., 2008). Many of these compounds are not identified, raising concerns about safety and health effects of water treatment methods (Richardson et al., 2008 & 2007). Schenck et al., (2009) demonstrated a dose dependent correlation between the mutagenicity and chlorination of the drinking water. The nature and concentrations of disinfection by-products are mainly influenced by type of disinfectant used and also by the distribution time of the treated water (reviewed by Bull et al., 2009).

Chlorination, the most common disinfection method, forms by-products including trihalomethanes (THMs), chloroform, bromodichloromethane (BDCM), chlorodibromomethane (CDBM), and bromoform. Some of these contaminants are teratogenic to laboratory animals. Haloacetic acids, at concentrations higher than found in tap water, cause NTDs in rat embryos (Ahmed et al., 2005, Andrews et al., 2004; Hunter et al., 1996; Hunter et al., 2006). Ahmed et al., (2005) demonstrated that exposure to chloroacetonitrile (CAN), another disinfection by-product, causes degeneration of neurons affecting the development of fetal brain in CD-1 mice. Additionally, CAN crosses the placenta to induce oxidative stress and apoptosis in the fetal brain. Epidemiological data on reproductive effects of chlorination disinfectant by-products indicate that these products can cause birth defects such as low birth weight, skeletal deformities, growth retardation, spontaneous abortions and NTDs but at relatively low rates (reviewed by Bove et al., 2002). In general, it has been accepted that health risks from exposure to disinfection byproducts (DBPs) are less than from consuming untreated water.

Petroleum products such as diesel, gasoline and kerosene are obtained by refining crude oil, natural gas and coal. Various storage, handling and transport conditions required for the extensive use of petroleum products provides opportunities for spills. Petroleum products may be released into the environment because of leaks from storage tanks and pipes, overfills, spills or intentional disposal. These products can travel through soil and contaminate drinking water sources. Petroleum products contain potentially toxic compounds such as xylene and toluene, and also contain additives such as lead and methyl tertiary-butyl ether (MTBE) which have various adverse health effects. Some of these compounds are known to be teratogenic. Xylene causes low birth weight, cleft palate and other skeletal deformities (Marks et al., 1982; Saillenfait et al., 2003). Toluene is teratogenic and also reacts synergistically with acetylsalicylic acid and other compounds that may be present in the water (reviewed by Wilkins-Haug, 1997).

In this study we investigated the possible cause of unknown NTDs in mice. The presence of NTDs in embryos of time pregnant mice obtained from different vendors indicate that the NTDs were not isolated to our facility and also that they were not localized to a single supplier or strain of mouse. Coupled with results on serology and housing type, it would seem that an infectious or genetic cause for these NTDs is unlikely. In mice, it is known that the maternal diet alters the risk of NTD development in embryos (Hariss and Juriloff, 2005; Stoate et al., 2008); however, in our study the diet has no significant effect on NTDs in newly purchased mice, thus excluding diet as the cause of these unknown NTDs. The incidence of NTDs was decreased when newly purchased mice were provided DDI or HPLC water for 2 weeks, but was not completely eliminated. Observation of NTDs in commercially obtained timed pregnant mice (Figure 3) indicates that the mice were possibly exposed to a teratogen prior to purchase. We hypothesized that the residual effect of teratogen due to a long half life may be responsible for the low levels of NTDs in purchased mice that were provided DDI or HPLC water. This was confirmed by providing the mice with DDI water for 2 generations where no NTDs were observed in offspring of F2-DDI mice.

Since NTDs were not observed in F2-DDI mice or in F2-DDI mice transferred to HPLC water, but reappeared when F2-DDI mice were exposed to tap water from different municipalities, it can be concluded that the cause of these unknown NTDs is exposure to tap water. The higher rates of NTDs in mice exposed for 8 weeks vs. 2 weeks, indicates a dose effect of some contaminant present in tap water. This dose response may account for the observed differences in NTD rates in the time pregnant mice (Figure 4) indicating varying lengths of tap water exposure and not necessarily a strain or location effect. Different strains, however, may show variable vulnerability to teratogens because of their difference in genetic background (Burren et al., 2009; Ogawa et al., 2005; Lemos et al., 2009). Our data suggests that the teratogen apparently has wide geographic distribution as mice from different regions of the country exhibited NTDs.

In this study, we demonstrated an association between tap water and NTDs in mouse embryos. This the first study to show fetal malformations from ambient exposure to tap water. There is evidence that the teratogen has a wide spread geographic distribution as mice exposed to tap water from 3 different locations, and mice purchased from NY, NC and MI all demonstrated NTDs. Specific seasonal variation in NTDs incidence was not tested; however, we did not notice any fluctuations in rates of NTDs throughout the 2 years working on this project. Published literature indicates that many teratogenic compounds can be found in drinking water but most are found below toxic concentrations. The teratogen causing NTDs in our mice has not yet been identified. Water analysis is currently underway to identify the candidate contaminants that might be responsible for the malformations.

Acknowledgments

Supported by NIH grant K01RR16241-01; Harvey Peter's Foundation; Institute for Critical Technology and Applied Science (ICTAS)

References

- Ahmed AE, Jacob S, Campbell GA, Harirah HM, Perez-Polo JR, KMJ. Fetal origin of adverse pregnancy outcome: the water disinfectant by-product chloroacetonitrile induces oxidative stress and apoptosis in mouse fetal brain. *Brain Res Dev Brain Res*. 2005; 159(1):1–11.
- Andrews JE, Nichols HP, Schmid JE, Mole LM, Hunter ES 3rd, Klinefelter GR. Developmental toxicity of mixtures: the water disinfection by-products dichloro-, dibromo- and bromochloro acetic acid in rat embryo culture. *Reprod Toxicol*. 2004; 19(1):111–116. [PubMed: 15336719]
- Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environ Sci Technol*. 2009; 43(3):597–603. [PubMed: 19244989]
- Bove F, Shim Y, Zeitz P. Drinking water contaminants and adverse pregnancy outcomes: a review. *Environ Health Perspect*. 2002; 110(Suppl 1):61–74. [PubMed: 11834464]
- Bull RJ, Rice G, Teuschler LK. Determinants of whether or not mixtures of disinfection by-products are similar. *J Toxicol Environ Health A*. 2009; 72(7):437–60. [PubMed: 19267306]
- Burren KA, Scott JM, Copp AJ, Greene ND. The genetic background of the curly tail strain confers susceptibility to folate-deficiency-induced exencephaly. *Birth Defects Res A Clin Mol Teratol*. 2009 (In press).
- Cabrera RM, Hill DS, Etheredge AJ, Finnell RH. Investigations into the etiology of neural tube defects. *Birth Defects Res C Embryo Today*. 2004; 72(4):330–344. [PubMed: 15662706]
- Christianson, A.; Howson, CP.; Model, B. March of Dimes Global Report, Executive Summary: The hidden toll of dying and disabled children. March of Dimes Foundation; White Plains, NY: 2006.
- Falconer IR. Are Endocrine Disrupting Compounds a Health Risk in Drinking Water? *Int J Environ Res Public Health*. 2006; 3(2):180–184. [PubMed: 16823090]
- Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology*. 2006; 76(2):122–159. [PubMed: 16257063]
- Glassmeyer ST, Hinchey EK, Boehme SE, Daughton CG, Ruhoy IS, Conerly O, Daniels RL, Lauer L, McCarthy M, Nettesheim TG, Sykes K, Thompson VG. Disposal practices for unwanted residential medications in the United States. *Environ Int*. 2008 (In press).
- Harris MJ, Juriloff DM. Mini-review: toward understanding mechanisms of genetic neural tube defects in mice. *Teratology*. 1999; 60(5):292–305. [PubMed: 10525207]
- Harris MJ, Juriloff DM. Maternal diet alters exencephaly frequency in SELH/Bc strain mouse embryos. *Birth Defects Res A Clin Mol Teratol*. 2005; 73(8):532–40. [PubMed: 15968625]
- Hunter ES 3rd, Blanton MR, Rogers EH, Leonard Mole M, Andrews J, Chernoff N. Short-term exposures to dihaloacetic acids produce dysmorphogenesis in mouse conceptuses in vitro. *Reprod Toxicol*. 2006; 22(3):443–448. [PubMed: 16527447]
- Hunter ES 3rd, Rogers EH, Schmid JE, Richard A. Comparative effects of haloacetic acids in whole embryo culture. *Teratology*. 1996; 54(2):57–64. [PubMed: 8948541]
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance. *Environ Sci Technol*. 2002; 36(6):1202–1211. [PubMed: 11944670]
- Lemos MC, Harding B, Reed AA, Jeyabalan J, Walls GV, Bowl MR, Sharpe J, Wedden S, Moss JE, Ross A, Davidson D, Thakker RV. Genetic background influences embryonic lethality and the occurrence of neural tube defects in *Men1* null mice: relevance to genetic modifiers. *J Endocrinol*. 2009; 203(1):133–42. [PubMed: 19587266]
- Marasas WF, Riley RT, Hendricks KA, Stevens VL, Sadler TW, Gelineau-van Waes J, Missmer SA, Cabrera J, Torres O, Gelderblom WC, Allegood J, Martinez C, Maddox J, Miller JD, Starr L, Sullards MC, Roman AV, Voss KA, Wang E, Merrill AH Jr. Fumonisin disrupt sphingolipid

- metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *J Nutr.* 2004; 134(4):711–716. [PubMed: 15051815]
- Marks TA, Ledoux TA, Moore JA. Teratogenicity of a commercial xylene mixture in the mouse. *J Toxicol Environ Health A.* 1982; 9(1):97–105.
- Munger R, Isacson P, Hu S, Burns T, Hanson J, Lynch CF, Cherryholmes K, Van Dorpe P, Hausler WJ Jr. Intrauterine growth retardation in Iowa communities with herbicide-contaminated drinking water supplies. *Environ Health Perspect.* 1997; 105(3):308–14. [PubMed: 9171992]
- Ochoa-Acuña H, Frankenberger J, Hahn L, Carbajo C. Drinking-water herbicide exposure in Indiana and prevalence of small-for-gestational-age and preterm delivery. *Environ Health Perspect.* 2009; 117(10):1619–24. [PubMed: 20019915]
- Ogawa T, Kuwagata M, Ruiz J, Zhou FC. Differential teratogenic effect of alcohol on embryonic development between C57BL/6 and DBA/2 mice: a new view. *Alcohol Clin Exp Res.* 2005; 29(5):855–63. [PubMed: 15897731]
- Rice G, Teuschler LK, Speth TF, Richardson SD, Miltner RJ, Schenck KM, Gennings C, Hunter ES 3rd, Narotsky MG, Simmons JE. Integrated disinfection by-products research: assessing reproductive and developmental risks posed by complex disinfection by-product mixtures. *J Toxicol Environ Health A.* 2008; 71(17):1222–34. [PubMed: 18636394]
- Richardson SD, Thruston AD Jr, Krasner SW, Weinberg HS, Miltner RJ, Schenck KM, Narotsky MG, McKague AB, Simmons JE. Integrated disinfection by-products mixtures research: comprehensive characterization of water concentrates prepared from chlorinated and ozonated/postchlorinated drinking water. *J Toxicol Environ Health A.* 2008; 71(17):1165–86. [PubMed: 18636390]
- Richardson SD, Plewa MJ, Wagner ED, Schoeny R, Demarini DM. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat Res.* 2007; 636(1–3):178–242. [PubMed: 17980649]
- Saillenfait AM, Gallissot F, Morel G, Bonnet P. Developmental toxicities of ethylbenzene, ortho-, meta-, para-xylene and technical xylene in rats following inhalation exposure. *Food Chem Toxicol.* 2003; 41(3):415–429. [PubMed: 12504174]
- Schenck KM, Sivaganesan M, Rice GE. Correlations of water quality parameters with mutagenicity of chlorinated drinking water samples. *J Toxicol Environ Health A.* 2009; 72(7):461–7. [PubMed: 19267307]
- Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA, von Gunten U, Wehrli B. The challenge of micropollutants in aquatic systems. *Science.* 2006; 313 (5790):1072–1077. [PubMed: 16931750]
- Snyder SA, Benotti MJ. Endocrine disruptors and pharmaceuticals: implications for water sustainability. *Water Sci Technol.* 2010; 61(1):145–54. [PubMed: 20057100]
- Stackelberg PE, Furlong ET, Meyer MT, Zaugg SD, Henderson AK, Reissman DB. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. *Science of The Total Environment.* 2004; 329(1–3):99–113. [PubMed: 15262161]
- Stackelberg PE, Gibb J, Furlong ET, Meyer MT, Zaugg SD, Lippincott RL. Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. *Science of The Total Environment.* 2007; 377(2–3):255–272. [PubMed: 17363035]
- Stoate KL, Harris MJ, Juriloff DM. Accelerated embryonic development associated with increased risk of neural tube defects induced by maternal diet in offspring of SELH/Bc mice. *Birth Defects Res A Clin Mol Teratol.* 2008; 82(10):720–727. [PubMed: 18798560]
- United States- Environmental Protection Agency (US-EPA). Factoids: Drinking Water and Ground Water Statistics for 2008. 2008 EPA 816-K-08-004.
- Villanueva CM, Durand G, Coutté MB, Chevrier C, Cordier S. Atrazine in municipal drinking water and risk of low birth weight, preterm delivery, and small-for-gestational-age status. *Occup Environ Med.* 2005; 62(6):400–5. [PubMed: 15901888]
- Wilkins-Haug L. Teratogen update: toluene. *Teratology.* 1997; 55(2):145–151. [PubMed: 9143096]

Figure 1.
Breeding scheme of mice to obtain embryos from mice provided with DDI water for 2 generations (F2-DDI).

Figure 2.

NTDs in late day 9 to day 13 mouse embryos caused by tap water. A–C. Normal day 9 embryos, 3 different views; D. Day 9 embryo with unfused neural folds at initiation site 3 resulting in a split face defect; E–F. Day 9 embryo with unfused neural folds in the midbrain region resulting in classic exencephaly; G. Day 9 embryo with unfused neural folds at both initiation sites 2 and 3 resulting in exencephaly and a split face; H. Day 10 embryo with unfused neural folds rostral to initiation site 1, resulting in spina bifida; I. Day 13 embryo with classic exencephaly caused by failure of the neural folds to fuse in the midbrain region.

Figure 3.

Association of type of housing and NTDs in CD-1 mice. There was no significant difference in NTD rates in mice housed in conventional open mouse boxes, under sterile conditions in a HEPA filtered laminar flow hood, and in a separate facility under BSL2 containment conditions. Values represent the mean percentages $\pm$ SE of fetuses affected with NTDs. (n=92 litters for conventional; n=9 litters in Hood; n = 3 litters in BSL2). Statistical significance was set at $p \leq 0.05$

Figure 4. Incidence of NTDs in timed pregnant mice from 3 different commercial suppliers in 4 different geographical locations. Values represent the mean percentages $\pm$ SE of fetuses affected with NTDs from 8–9 litters per group. Statistical significance was set at $p \leq 0.05$

Figure 5.

Association of type of diet and NTDs in CD-1 mice. There was no significant difference in NTD rates in mice fed Teklad 7013 diet, Ziegler diet NTP-2000, Zeigler 18-6 diet or Purina 5001. Values represent the mean percentages $\pm$ SE of fetuses affected with NTDs. (n = 9 litters in Zeigler NTP-2000; n=61 litters in Zeigler 18:6; n=3 litters for Teklad 7013; Diet; n=31 litters in Purina 5001). Statistical significance was set at $p \leq 0.05$

Figure 6.

Association of type of water and neural tube defects in purchased CD-1 mice. NTD rates were significantly decreased in mice that were provided DDI or HPLC water compared to tap water. Values represent the mean percentages $\pm$ SE of fetuses affected with NTDs. (n = 25 litters in Tap; n=30 litters in DDI water; n=26 litters for HPLC water). Statistical significance was set at $p \leq 0.05$

Figure 7.

NTDs in offspring of dams maintained exclusively on DDI for 2 generations (F2-DDI) and switched to municipal tap water 2 or 8 weeks prior to breeding. Mice exposed to tap water for 8 weeks had significantly higher rates of NTDs in their embryos than mice exposed to DDI water and tap water for 2 weeks. Values represent the mean percentages $\pm$ SE of fetuses affected with neural tube defects. $n=7-9$ litters per group in the 2 week exposure, and $14-15$ litters per group in the 8 week exposure. Statistical significance was set at $p \leq 0.05$