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Pharmaceuticals and other Organic Wastewater Compounds in Arizona's Effluent and Implications for Water Reuse by *Gail E. Cordy*

Treated effluent is a valuable resource in Arizona and the Southwest where it is used to recharge dwindling ground-water supplies and sustain agricultural and urban irrigation, wetlands, and riparian areas. Recent studies of pharmaceuticals, hormones, and other organic wastewater compounds (OWCs) in Arizona's streams (Kolpin and others, 2002) indicate that some of these compounds are not removed during the wastewater-treatment process, but are being discharged into streams. Some pathogens may also survive the treatment process (Toze, 1999).

A proof-of-concept experiment was devised by researchers from the USDA Water Conservation Laboratory and the U.S. Geological Survey (USGS) (Cordy and others, 2004) to determine if pharmaceuticals, other organic wastewater compounds (OWCs), and selected pathogens in treated effluent could be transported through a soil column under ground-water recharge conditions similar to those in arid or semiarid climates. Treated effluent was applied at the top of the 2.4-m long, 32.5-cm diameter soil column over 23 days. Samples of the column inflow were collected from the effluent storage tank at the beginning and end of the experiment, and a sample of the soil-column drainage at the base of the column was collected at the end of the experiment. Samples were analyzed for 131 OWCs including veterinary and human antibiotics, other prescription and nonprescription drugs, widely used household and industrial chemicals, and steroids and reproductive hormones as well as the pathogens, *Salmonella* and *Legionella*.

Analytical results for the two effluent samples taken from the effluent storage tank at the beginning and end of the experiment indicate that the number of OWCs detected in the column inflow decreased by 25% (8 compounds), and the total concentration of OWCs decreased by 46% while the effluent was in the storage tank for 23 days. After percolating through the soil column, an additional 18 compounds detected in the effluent storage tank at the end of the experiment (67% of OWCs) were no longer detected in the soil-column drainage, and the total concentration of OWCs decreased by more than 70%. These compounds may have been subject to transformation (biotic and abiotic), adsorption, and (or) volatilization in the storage tank and during travel through the soil column. Eight compounds— carbamazepine; sulfamethoxazole; benzophenone; 5-methyl-1H-benzotriazole; N, N-diethyltoluamide; tributylphosphate; tri(2-chloroethyl) phosphate; and cholesterol—were detected in all three samples indicating that they have the potential to reach ground water under recharge conditions. Results from real-time polymerase chain reactions demonstrated the presence of *Legionella* in all three samples. *Salmonella* was detected only in the effluent at the beginning of the experiment, suggesting that the bacteria died off in the effluent storage tank over the period of the experiment. This proof-of-concept experiment demonstrates that, under recharge conditions similar to those in arid or semiarid climates, some pharmaceuticals, pathogens, and other OWCs can persist in treated effluent after soil-aquifer treatment.

With support from the U.S. Environmental Protection Agency (USEPA), staff from the USGS and the USDA, Water Conservation Laboratory, developed a study to determine the temporal occurrence and persistence of pharmaceuticals, pathogens, and OWCs in an effluent-dependent stream. Over a 24-hour period coinciding with low, rising, peak (2), and falling streamflow at each of four sites, streamflow in the Santa Cruz River near Tucson was sampled. Sampling sites included the Roger Road Wastewater Treatment Plant (WWTP) outfall, the Ina Road WWTP flume (6.8 km downstream), the Santa Cruz River at Cortaro Road (2.9 km downstream from the Ina Road WWTP flume) and the Santa Cruz River at Trico Road (11.4 km downstream from the Cortaro Road site). Samples were analyzed for 102 compounds including prescription and non-prescription drugs and other OWCs by methods 3 and 4, respectively, as described in Kolpin and others (2002). The presence of several bacterial indicators was determined by polymerase chain-reaction analyses.

The number of compounds (44) detected in the Roger Road WWTP outfall remained relatively stable over 24 hours and did not appear to be influenced by the discharge; whereas, the number of compounds in effluent from the Ina Road WWTP flume varied from 36 at low flow to

a high of 47 compounds in rising flow. The number of compounds detected in flow at Cortaro Road was highest (45) at low flow and lowest at peak flows (40 and 38 compounds) indicating that dilution may be an important factor at this site where the Roger Road and Ina Road WWTP flows coalesce. At the Trico Road site, fewer compounds were detected at all flows compared to all other samples collected at the three upstream sites. This is not unexpected given that some compounds can be lost from the streamflow through photolysis, volatilization, or adsorption to sediment and organic material. Additionally, during transport downstream, some compounds may biodegrade or be transformed to metabolites that were not analyzed as a part of this study and, thus, were not detected. The number of compounds at Trico Road was smallest (26) during decreasing (falling) streamflow and highest (35) during rising flow. Compounds that persisted farthest downstream included an insect repellent, detergent metabolites, fire retardants, an antimicrobial agent, cholesterol, as well as various human drugs including antibiotics, a nicotine metabolite, an antiepileptic, an antacid, an antidepressant, an antiasthmatic, an analgesic, an antihypertensive, an antianginal, an antifungal, and caffeine and its metabolite.

Results of the temporal sampling demonstrated that coliform numbers varied considerably during the 24-hour period. There was a correlation between bacterial numbers and discharge at the Ina Road WWTP flume. Coliform numbers were highest (800 CFU/100 mL) in the evening (21:00) during the second peak flow and lowest (100 CFU/100 mL) in the early morning (06:00) during lowest flow. These results emphasize the need for composite daily load measurements rather than a single daily measurement near the point of discharge in order to get a more accurate assessment of microbial inputs from effluent discharge into streams.

A recent collaboration with Dr. David Walker, University of Arizona, and the USGS on a study of the effects of treated wastewater on native fish yielded results similar to those noted in other studies. Analyses of fish blood hormones showed that exposed male fish had 17-beta estradiol (female hormone) levels that were significantly higher and 11-ketotestosterone (male hormone) levels that were significantly lower than control males. In addition, exposed males had higher concentrations of the egg protein, vitellogenin, than control males. These data indicate a feminization of male fish from effluent exposure. Treatment effects in female fish were not as pronounced as those for males.

The persistence of OWCs and indicator bacteria during all flow regimes in the Santa Cruz River as well as the soil-column study results indicating the persistence of some OWCS and *Legionella* during recharge raises concerns about the potential effects of reuse and recharge of effluent from the Santa Cruz River on ground-water quality. Very few of the OWCs detected in these studies are currently regulated by the USEPA for drinking water, in part, because the human health effects of low concentrations of individual OWCs and mixtures are difficult, if not impossible to determine. Given the known effects of endocrine-disrupting compounds in effluent on aquatic life, municipalities recharging treated wastewater should take a conservative approach to recharge of effluent for drinking-water purposes. At some point, additional treatment to remove persistent OWCs may need to be considered.

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New Disinfection By Products

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EMERGING CONTAMINANTS WORKSHOP

Maricopa Cooperative Extension Office

4341 East Broadway Road, Phoenix, AZ March 2, 2007

New DBP Categories

- **Non-Halogenated nitrogenous DBPs**
- **Halogenated nitrogenous DBPs**
- **Halogenated carbonaceous DBPs**
- **Iodinated DBPs**

Why worry about some of the Recently Identified DBPs ?

- **Brominated and iodinated DBPs are more potent toxins compared to their chlorinated counterparts (Woo et al., 2002)**
- **Certain nitrogenous DBPs are more cytotoxic and genotoxic than some of the regulated carbonaceous DBPs (Plewa et al., miscellaneous publications)**

Some Concluding Remarks

- **Emergence of new DBPs may alter the perspective of health effects studies significantly**
- **Historically health effects studies primarily considered carbonaceous DBPs, total organic carbon and bromide**
- **New DBPs discussed in this presentation and their toxic potency is leading health effects studies that focus on organic nitrogen and wastewater derived organic matter**
- **Treatment technologies that address regulated and emerging DBPs should be considered by the water industry**

“Detection and Fate of Nanomaterials During Water and Wastewater Treatment”
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Technologic revolutions bring great benefits to society. Unfortunately, most also result in residuals that can have detrimental impacts on human health and the environment. A case in point is the widely-published environmental issues associated with trace-level pharmaceuticals and personal care products (PPCPs), which evolved out of the biotechnology revolution of the 1980s and 1990s. PPCPs will remain a challenge, but today we are on the cusp of a new nanotechnology revolution catalyzed by advances in surface characterization and material fabrication at the nanometer scale (10^{-9} meter). Because of their size (<100 nm) and unique properties (e.g., surface area, quantum effects, molecular recognition), nanomaterials (NMs) are resulting in new medical, industrial and commercial products. American (USEPA, NIEHS), European, and Japanese governments have expressed concern regarding the impacts on society and the environment from NMs and their manufacture. Because much of society’s residuals will pass into the environment through more than 17,000 U.S. wastewater treatment systems (by 2016 these systems will serve more than 90% of the U.S. population), NMs will impact wastewater treatment plants (WWTPs) just as PPCPs have over the past decade. We need to recognize the new and potential impacts of nanomaterials at WWTPs today. Let’s not wait five or ten years before find nanomaterials ubiquitously in river.

Commercial nanomaterials (CNMs) are increasingly likely to be toxic to humans and ecosystems as the nanotechnology revolution evolves. However, little scientific information is currently available on the fate of CNMs in WWTPs, whether they are present in biosolids or effluent, or the potential impact of CNMs on the treatment processes. CNMs are used in 200-plus consumer products today: stain-resistant clothing, sunscreens, air fresheners, cosmetics, storage bags, pain relief cream. While this emerging technology brings advanced products and scientific advances to humanity – including use for drug delivery and treatment – little is known about its impact on human health, the environment and ecosystems.

Pefluorocarbons in the Environment: Fate, Persistence and Remediation Options

EMERGING CONTAMINANTS WORKSHOP

Trace Chemical Contaminants in Water and Wastewater – Semiarid Perspectives Phoenix, March 2, 2007

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Heavily fluorinated polymers and telomers (short-chain polymers) are widely used in the manufacture of well-known products such as Teflon, Stainmaster, Scotchgard and Gore-Tex, which are intended to be chemically inert and to resist wetting or staining. These compounds, referred to as PFCs, are also used in shampoos, floor waxes, cleaning products, non-stick cookware, and to coat paper food wraps and fast food containers. Teflon itself is an example of a perfluorinated polymer. Nearly all PFCs and their parent compounds eventually degrade into a family of extremely stable end products that are closely related to perfluorooctanoic acid (PFOA) or perfluorooctanesulfonic acid (PFOS). These degradation compounds most commonly occur as salts, not as the free acid, and appear to be entirely inert in the environment, being immune to biodegradation, direct solar photolysis, indirect photolysis in the presence of hydrogen peroxide or a Fenton reagent, and to hydrolysis. As a result, they and their related telomers have been steadily accumulating in the environment since they first began to be manufactured about 40 years ago. They have been found in waste water, groundwater, surface water, and tap water, in soils and in the air. Recent evidence suggests that they are unaffected though wastewater treatment plants. It is thought that airborne PFCs originate from a relatively volatile fluorinated alcohol, that can be widely dispersed through the atmosphere, and which ultimately degrades to PFOA. A recent study has determined that residual PFOA remains in Teflon products such as non-stick cookware after manufacture, and that it can be released at typical cooking temperatures. PFCs have also been detected in animals and plants across the globe, and they are present in the entire human food chain, including beans, apples, beef and bread. All seven members of the PFOA family and all eight members of the PFOS family have been detected in human blood. PFOA and PFOS are detected more frequently in children than in adults. The half-life of PFCs in humans is thought to range from 1 to 9 years, with most estimates converging on approximately 4 years.

PFOS and related compounds are used extensively in the semiconductor industry, principally in wafer photolithography, where PFOS is used as a surfactant, a photoacid generator, and a precursor of antireflective coating, at concentrations that can reach 1%. There is evidence that PFOS may be present in wastewater from photolithografic facilities. Nevertheless, there are no known compounds that can act as PFOS substitutes in this type of process.

Health concerns about PFCs arose only recently because of new observations that PFOA can harm laboratory animals at blood levels *below* those already found in some children. For

example, in a rat reproduction study, embryos were exposed *in utero* to maternal blood levels of 40-1000 ppb PFOA. At 40 ppb, effects included low birth weight, decreased pituitary size, decreased liver size, and a 10% excess mortality rate. No excess mortality was seen in adult rats at this dose, indicating a greater susceptibility of infants to PFOA. Mean PFOS serum levels in the general U.S. population are approximately 43 ppb. In American children the mean PFOA blood level is 5.6 ppb but the range extends up to 56 ppb. The PFOA blood levels in production workers at 3M ranged from 1000 to 114,000 ppb. Longitudinal studies of these and other workers suggest that PFOA may cause testicular, breast, liver and prostate tumors. It may also cause hypothyroidism and hormonal imbalances in men (elevated estrogen levels and abnormal testosterone regulation). As a result, the EPA now lists PFOA as a carcinogen in animals, and it has initiated a priority review of PFOA with a view to regulating it under the Toxic Substance Control Act (TSCA). The European Union is proposing legislation to ban the use of PFOS completely.

The strength of the C-F bond combined with the high electronegativity of fluorine make PFOA and PFOS exceptionally stable chemical compounds that are highly resistant to degradation, often by design. The strength of the C-F bond exceeds that of the H-H bond, so simple chemical reduction is unlikely to be effective, even if the process was to be catalyzed somehow. Oxidation is also thermodynamically unfavorable. It is not surprising that hydrogen peroxide and the hydroxyl radical are ineffective reagents, and that enzymatic action is typically ineffective. Because of this, conventional treatment strategies have failed and so unusual and/or expensive approaches may be required. For example, there is evidence that the C-F bond can be broken by ultrasonic irradiation where degradation occurs via high temperature pyrolysis in cavitation bubbles. Other recalcitrant pollutants have been treated by radiolysis (gamma rays or X-rays), or by high-energy UV irradiation (UV-C). Ultimately, it may be necessary to concentrate PFCs prior to their destruction by first extracting them from contaminated groundwater using granular activated carbon, or other appropriate sorbent. Current research at the University of Arizona is exploring the use of radiolysis, biodegradation and electrocatalysis as possible pathways for remediation of water containing PFCs.

Estrogens and PBDEs in Wastewater and Sludge

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Conventionally treated wastewater contains a myriad of organic contaminants in trace amounts. Most of these may be innocuous from the perspectives of human and environmental health. A few such compounds, however, are bioactive at exceptionally low concentrations, on the order of a few parts per trillion. Hormones and hormone mimics, also referred to as endocrine disrupting compounds (EDCs), are particularly troubling in this regard. For this reason, our research focuses on EDCs. In particular, we have examined the fate of residual estrogenic compounds and polybrominated diphenyl ethers (PBDEs) in wastewater and sludge during treatment processes comprising conventional wastewater treatment, polishing operations (e.g. soil aquifer treatment), river transport, and land application of biosolids.

Estrogens such as 17 β -estradiol (E₂), 17 α -ethinylestradiol (EE₂), estrone (E₁), estriol (E₃), and estrogen mimics such as nonylphenol, octylphenol, bisphenol A, etc., are present in all municipal wastewaters and survive, to a degree, during conventional wastewater treatment. Estrogens and estrogen mimics in treated wastewater are probably responsible for elevated incidence of intersex characteristics among fish and other animals, particularly those exposed during growth and developmental phases of life.

Approaches to measurement of EDCs fall in one of two categories: direct chemical measurement of individual compounds via GC-MS or LC-MS; or indirect collective measurement of endocrine disruption activity via cell-based (*in vitro*) or whole-animal-based (*in vivo*) bioassays. A number of *in vitro* assays are available to measure whole-sample estrogenic activity. Our research group utilizes two techniques to evaluate fate of estrogenic activity during wastewater treatment and reclamation processes: the yeast estrogen screen (YES) and a reporter gene assay using a human breast cancer cell line (T47D) that was recently developed by the USEPA.

Measurement of estrogenic activity in chemically complex water samples such as treated wastewater is particularly challenging due to the potential presence of estrogens and anti-estrogenic compounds. The mechanism of anti-estrogenic activity remains to be determined. It is significant that estrogens and anti-estrogens can be present in the same samples.

Recent work at a series of Arizona wastewater treatment plants (WWTPs) showed significant differences in the efficiencies of estrogenic activity attenuation (Table 1). Effluent estrogenic activity from the biotower #1 facility was > 300x the level in WWTP effluent from a nitrification/denitrification facility. The effluent concentration from the biotower #1 facility was equivalent to the influent level of estrogenic activity at many of the plants sampled and higher than EE₂ concentrations known to produce developmental problems in continuously exposed fish.

Table 1. Total estrogenic activities at several municipal wastewater treatment facilities in Arizona. Influent and effluent EE₂-EQ values were obtained using the YES bioassay.

Total Estrogenic Activity (M as EE ₂)			
<i>Facility Type</i>	<i>Influent</i>	<i>Effluent</i>	<i>Fraction Removal</i>
Oxidation Ditch	1×10^{-9}	2×10^{-12}	0.998
Biotower#1	3×10^{-10}	2×10^{-10}	0.33
Activated sludge (pure O ₂)	4×10^{-11}	5×10^{-12}	0.88
Membrane bioreactor	2×10^{-10}	$< 2 \times 10^{-12}$	>0.99
Biotower#2	1×10^{-10}	4×10^{-12}	0.96
Nitrification/denitrification	1.5×10^{-10}	$< 6 \times 10^{-13}$	>0.996

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Both natural and managed infiltration of secondary effluent are effective barriers to EDC transport to groundwater. There is a significant degree of attenuation of the estrogenic signal in treated wastewater during infiltration through unconsolidated sediments. The majority of that attenuation occurs in the top meter (or less) of sediments, so that great depth to groundwater is unnecessary to achieve this type of water quality benefit.

Estrogenic activity decreased substantially (75+%) during in-stream travel (1-2 days) in the effluent dependent Santa Cruz River near Tucson. River waters are typically shallow and relatively warm – conditions that may promote chemical and biochemical transformations of residual estrogenic compounds in wastewater effluent.

Constructed wetland treatment appears modestly effective in removing estrogenic activity from secondary effluent. Removals of about 60% were observed during passage through a full-scale free-water-surface wetland. During subsurface-flow wetland treatment at the Constructed Ecosystems Research Facility (CERF), removal of estrogenic activity was season dependent with less removal occurring during the hottest months of the year.

Recently it was discovered that measurable amounts of polybrominated diphenyl ethers (PBDEs) are present in biosolids derived from sludge digestion processes and at lesser levels in wastewater effluents. PBDEs are a class of flame retardants commonly added to consumer products. They are suspected endocrine disruptors, affecting both the estrogen and thyroid hormone systems. Their concentrations in human tissues have increased rapidly over the last few decades with a doubling time on the order of five years.

PBDE were detected in surface soils at land application test plots receiving biosolids in Arizona and Washington State. Concentrations of total PBDEs in these soils were on the order of a few hundred μg per kg dry soil and decreased by about an order of magnitude at twelve-inch depths below the surface. A mass balance calculation approach performed at the Arizona site suggests that PBDEs accumulate over time and are not readily degraded in soil.

We have also found PBDEs in surface sediments of infiltration basins receiving secondary effluent. Their long-term fate in those sediments is a matter of current interest. Possibilities include accumulation on sediments with potential for eventual breakthrough to ground water, relatively slow biodegradation or eventual incorporation into soil humus. Our data show that sediment PBDE content decreases rapidly in the first 20 cm of depth. The highest sediment concentration observed in local infiltration basins that have been in operation for 18 years was 383 μg PBDEs per kg of dry sediment. Concentrations at a depth of 100 cm were on the order of 10 $\mu\text{g}/\text{kg}$. Measurable concentrations of PBDEs were still present in water samples taken at a depth of 5 meters below the surface of an infiltration basin.

Preliminary work has shown the potential for uptake of PBDEs by plants growing at sites receiving either municipal biosolids or secondary effluent. Currently, this is an area of active research.

Catherine R. Propper. Disruption of Behavior and Development by Environmental Contaminants: from Single Compounds to Complex Mixes. Department of Biological Sciences, Northern Arizona University. Affiliate, Arizona Water Institute.

Wastewater effluent world-wide has demonstrated endocrine disrupting actions, and the physiological outcome of exposure to effluent on wildlife species includes development of gonadal abnormalities, changes in sexually dimorphic characteristics, and outright sex reversal. Many of these effects are mediated through the estrogenic-mimicking actions of the compounds released into the environment. However, some effects of physiological disruption by these compounds may be induced through non-estrogenic mechanisms. Little is understood about how these compounds impact behavior (via estrogenic or non-estrogenic means) and thyroid hormone-mediated development. Our research using amphibian model systems demonstrates that exposure to single compounds and wastewater containing complex mixes can impact behavior and/or development.

Many animals communicate reproductive state via olfactory mechanisms involving the secretion of chemicals that impact members of the opposite sex (pheromones). The production and detection of pheromones is under control of both the neural and endocrine systems. Using a salamander model system, we have demonstrated that exposure to a common pesticide, endosulfan, at levels found in the environment, disrupts pheromone production and detection, as well as reproductive behavior. Endosulfan is only weakly estrogenic, and the doses that disrupted behavior were below those found to be estrogenic suggesting this pesticide's actions were through a non-estrogenic mechanism.

The process of gonadal differentiation involves a complex cascade of gene expression events leading to the formation of either testes or ovaries. Fish and amphibians exposed to wastewater effluent in several parts of the world have demonstrated problems associated with gonadal differentiation. We have investigated the effects of exposure to a common wastewater pollutant, 4-tert-octylphenol (OP), on ovarian and testicular development using another amphibian model, the bullfrog (*Rana catesbeiana*). We found that 24 hours of exposure to environmental concentrations of OP both accelerates the process of gonadal formation and disrupts expression of a key gene involved in the formation of ovaries and testes. Even doses of OP below which it binds to the estrogen receptor were effective in disrupting expression of this key gene, suggesting that OP can impact gonadal development through an unidentified mechanism. This result demonstrates that low doses of a common contaminant can have biological effects on reproductive development, and that it is difficult to predict the effects of exposure based on estrogenicity estimates alone.

The thyroid hormone system is critical to development of the neural, endocrine, skeletal, muscular, and intestinal systems. Recent studies demonstrate that some individual compounds found in wastewater disrupt the thyroid hormone system. We have investigated the potential for wastewater to disrupt thyroid-mediated developmental processes using another amphibian model, the African clawed frog (*Xenopus laevis*). The process of undergoing amphibian metamorphosis (transition from tadpole to frog) is completely under the regulation of thyroid hormone. We have found that exposure to

wastewater during development accelerates metamorphosis, suggesting that compounds in wastewater either mimic thyroid hormone or induce natural thyroid hormone activity inappropriately.

Throughout the world, and especially in arid regions such as the United States Southwest, effluent is released into the environment. Human beings are exposed to these compounds through water and other resources such as food and air. The health-related outcomes of such exposure are uncertain, but wildlife studies suggest that there may be subtle, but real physiological impacts. Recent research in humans suggests that environmental chemical exposure is associated with effects on development, sperm count and quality, behavior, and increased risk of diabetes. This body of research suggests low dose exposure to “emerging contaminants” may influence the health of wildlife and humans.

As clean water resources diminish, use of wastewater effluent as reclaimed water for agriculture, recreation and aquifer recharge is critical. Therefore, it is important to define the impact of chemical mixes on development and adult function. Understanding the potential for physiological disruption by exposure to effluent is both a wildlife and human health imperative. Such research will determine the necessity for utilizing limited and competing public financial resources to develop and implement better water treatment strategies.

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The Ecological Effects of Endocrine Disruption; Quantification in a Threatened and Endangered Fish Species

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An individual organism's ability to exploit a resource (or group of resources) in the face of environmental stress and inter-specific competition, coupled with conservation of genetic material enabling this exploitation, is what drives speciation. Genetic conservation of traits is initiated, and sustained by, subtle behavioral cues for mating, spawning, aggression, territoriality, avoidance, etc. Any impairment of these behavioral cues or manifestation into physiological or morphological changes has the capability to stunt speciation by lowering fertility and fecundity. This type of endocrine-impairment is often alluded in aquatic systems receiving treated effluent of various kinds, but is rarely quantified due to the complexity of determining environmental cause and physiological effect. More often than not, endocrine-impairment of aquatic species is not readily morphologically evident and quantification can only be done by measuring relevant suites of biomarkers. Field-based studies lack the control and/or replication required for quantification of cause and effect whereas controlled experiments often suffer from a lack of ecological relevance. While it is never possible to satisfy both ends of this spectrum, research on endocrine-impairment of organisms should attempt to strike some degree of balance between mechanistic understanding and ecological relevance.

In this study, native bonytail chub (*Gila elegans*) were housed in large aquaria (with replicates and controls) and exposed to differing doses of treated effluent collected from the Santa Cruz River below Roger Rd. wastewater treatment plant. Each exposure period lasted 3 months. After each exposure, blood was collected from randomly-selected individuals within each control and treatment tank. Blood plasma samples were sent to the USGS Florida-Caribbean Science Center and analyzed for the sex hormones 17 β -estradiol, 11-ketotestosterone, and the egg protein vitellogenin. Gonadal histopathology was performed on 5 randomly selected fish from each raceway at the same time blood was drawn. Additionally, water samples were collected from treatment raceways at the end of each exposure and analyzed for over 90 organic compounds including antibiotics, prescription and non-prescription drugs, steroids and hormones, detergent metabolites, disinfectants, plasticizers, polyaromatic hydrocarbons, fire retardants, and pesticides. Analysis of water was performed by colleagues at the USGS National Water Quality Laboratory in Denver, Colorado.

Endocrine-impairment was evident at all doses but was most prominent in earlier exposures where concentrations of organic wastewater compounds were highest. Preliminary results indicate that sex hormones in water samples were below detectable limits. Due to the recirculating nature of the raceways, combined with passive filtration for nitrification, we anticipated that several non-persistent compounds would be degraded and our focus was on the most environmentally persistent compounds. At the landscape scale, the long-term, environmentally-persistent compounds are likely those causing the most harm ecologically. Those compounds readily degraded by oxidation, photolysis, or bacterial degradation, while harmful in their own right, primarily affect organisms living in close proximity to an outfall. We believe our experimental design more closely approximates conditions observed in the environment especially with distance from an outfall.

Differences in the primarily female sex hormone, 17 β -estradiol, were observed between treatment and control male fish during all treatments. The difference was most pronounced in

dose 1 where concentrations in treatment males (n = 5) were 5.7 times greater than controls (n = 2) (689 and 121 pg/ml respectively). For the primary male sex hormone in fish, 11-ketotestosterone, the greatest difference occurred during dose 1 where control (n = 2) males had 2.7 times the levels found in treatment (n = 5) males (1027 and 380 pg/ml respectively). Concentrations of the egg protein vitellogenin were much higher in treatment compared to control male fish for all doses. Similar to other hormone results, this response was the greatest in dose 1 where the mean concentration in treatment males (n = 5) was 15 times greater than control males (n = 2) (0.435 pg/ml and 0.029 pg/ml respectively). Treatment male fish had levels of 17 β -estradiol that approached or were often higher than control female fish. This same trend occurred with vitellogenin where treatment males had levels twice those of females for treatments 1 and 3.

Androgenization of female fish also occurred with the largest differences between treatment and control female fish observed in levels of 17 β -estradiol. Female fish during treatments 1 and 3 had essentially identical differences with controls having levels of 634 and 622 pg/mL respectively while treatment female fish had mean levels of 432 mg/L during both doses (n = 54 and 46 respectively)

Ratios of primary male and female hormones, in “undisturbed” populations would be expected to have an inverse relationship i.e. as one increased, the other would decrease. We could therefore assume that major deviations from this inverse relationship between male and female primary sex hormones, could be attributed to impairment.

Control female fish had concentrations of 17 β -estradiol which were inversely related to the concentration of 11-ketotestosterone (r = -0.67). Additionally, the control females exhibited an inverse relationship between the egg protein vitellogenin and 11-ketotestosterone (r = -0.70) and a positive relationship between 17 β -estradiol and vitellogenin (r = 0.52). This scenario changed drastically in the treatment female group with the strongly inverse relationship between 17 β -estradiol and 11-ketotestosterone noted in the control group, becoming slightly positive (r = 0.11). The strongly inverse relationship between 11-ketotestosterone and vitellogenin noted in control females was approximately half this amount in the treatment females (r = -0.36) and the positive relationship between 17 β -estradiol and vitellogenin was significantly reduced (r = 0.28).

Much like the control female group, control males exhibited an inverse relationship between 17 β -estradiol and 11-ketotestosterone (r = -0.89), vitellogenin and 11-ketotestosterone (r = -0.73), and a positive relationship between 17 β -estradiol and vitellogenin (r = 0.74). Also like the treatment females, treatment males exhibited a similar de-coupling of the inverse relationship between 17 β -estradiol and 11-ketotestosterone (r = -0.50) and between 11-ketotestosterone and vitellogenin (r = -0.28). The positive relationship between 17 β -estradiol and vitellogenin was only slightly less in the treatment males (r = 0.69).

While these results prove strong endocrine impairment in exposed fish, it does not quantify reproductive impairment. New and on-going work involves, once again, quantifying endocrine impairment in F₀ fish after exposure to treated effluent and then, by providing environmental cues for spawning, examining fertility and fecundity of treatment vs. control populations. Once this has been re-established, we will examine sex ratio and endocrine impairment of the F₁ generation. While much attention has focused on inter-sex fish found in the wild, impairment due to exposure of hormonally active compounds involves a cascade of events from environmental stimulus to the hypothalamus eventually leading to abnormal protein development and the inter-sex condition often observed. In this next study, we will examine biomarkers which take into account this temporal aspect of impairment including neuronal activity in the brain, aromatase (brain and ovarian), gonadotropins, and the sex hormones and proteins previously analyzed. We will also be working with colleagues at the USGS-Columbia Environmental Research Center using long-term *in-situ* sampling of water using polar organic chemical integrative samplers (POCIS) and semi-permeable membrane devices (SPMD's). This design is much more ecologically relevant than grab or point samples and are more representative of the body burden of exposed fish.

Modeling the Environmental Profiles of Stricken Communities in Mice

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When a community experiences an elevated rate of a particular disease and the unique contaminants to which the community is exposed are modeled in mice, the biological results of the exposure can be characterized and utilized to ascertain the role the exposure may play in the etiology of the disease. The exposure may affect expression of genes, such as tumor suppressers, which may act to increase the total number of people susceptible to developing the disease as compared with the control population, or affect expression of genes known to be directly related to the development of the diseased condition and therefore encourages the development of or directly precipitates the disease in those individuals already possessing a predisposition towards the disease.

Fallon, Nevada possesses elevated rates of childhood leukemia and is serving as a testing ground for these ideas. The causes of childhood leukemia are not known. The environmental exposures unique to Fallon include significantly elevated levels of tungsten and cobalt in the ambient particulate matter as well as slightly elevated levels of arsenic which also occurs in conjunction with a tungsten exposure in the childhood leukemia cluster in Sierra Vista, Arizona. We modeled these exposures in a two part investigation to determine their leukemogenic ability. When administered to young adult mice, tungsten did not cause a lymphoproliferative condition, but it did alter blood profiles during the first 63 days of exposure, with the most pronounced effect on the males but also inducing thrombocytopenia in both males and females. Because leukemia is hypothesized to occur prenatally, we exposed pups while *in utero* to tungsten, cobalt, arsenic, and tungsten/arsenic and tungsten/cobalt. We have conducted a gene microarray investigation with spleen tissue from pups exposed to tungsten/arsenic vs. controls and discovered transcriptome changes associated with leukemia specifically and carcinogenesis generally. Verifying the results with quantitative RT-PCR of the putative tumor suppresser gene, DMBT1 (Deleted in Malignant Brain Tumors 1), we found that tungsten decreased the expression of DMBT1 particularly in the population of mice that typically expressed this gene at higher levels. DMBT1 produces a protein product, gp340, which has been effectively utilized as a vaccine in tamarins to protect from B-cell lymphoma induced by the Epstein-Barr Virus. The effect of community wide exposure to tungsten on the expression of DMBT1 may serve as an example of how an exposure to a unique aspect of the environment may increase the number of individuals susceptible to a particular disease.

Ecologic Study of Communities to Discover Trace Contaminants

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The environmental sciences strategy of “ecologic” study (Sheppard et al. 2007e) around a community with a public health issue can help discover the presence of trace contaminants in the community, possibly leading to directed biomedical research to evaluate linkages between the contaminants and the disease. In ecologic study, the principal sampling unit of interest is the entire affected community, and other whole communities located in the same bioecoregion serve as the control or comparison groups. This strategy can result in definitive findings and conclusions about trace contaminants when the range of exposure to contamination is small (most everyone in the affected community is exposed to some degree) relative to a high range of individual susceptibility (some exposed people get sick, others do not).

Ecologic study differs from the traditional case-comparison study often done in public health investigations, where individual sick people within the affected community are the case subjects and other people within the same community who are not sick serve as comparison subjects. This strategy often results in no definitive conclusions about effects of contaminants on the disease affecting the community because even comparison subjects show evidence of exposure but they aren't sick.

Ecologic study was performed in Fallon, Nevada, which has experienced a cluster of childhood leukemia at a rate far greater than expected nationally. Fallon was the test subject, and nearby towns of west central Nevada as well as isolated locations in the surrounding desert itself were used as comparison subjects. Multiple lines of evidence were tested to corroborate across independent environmental monitoring and assessment techniques. First, total suspended particulates were filtered directly from air and measured for loading of trace metals, which show airborne tungsten and cobalt to be elevated in Fallon versus other communities (Sheppard et al. 2006). Second, lichen tissues were measured for concentrations of trace metals, and they also show tungsten and cobalt to be higher in Fallon than in outlying desert areas (Sheppard et al. 2007a). Third, surface dust was swept up from paved surfaces inside Fallon and measured for trace metals, and a local “hotspot” of elevated tungsten and cobalt is evident (Sheppard et al. 2007b). Fourth, tree-ring samples from trees growing in the hotspot were measured for trace metals, and tungsten began increasing there in the mid-1990s, about the same time as the onset of the cluster of childhood leukemia (Sheppard et al. 2007c). Last, a microanalysis of the tungsten particles themselves shows them to be anthropogenic in origin (Sheppard et al. 2007d).

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RESPIRATORY EFFECTS OF LOW-LEVEL ARSENIC EXPOSURE IN DRINKING WATER

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Arsenic is a naturally occurring metalloid found in water, soil and air. Exposure to inorganic arsenic occurs worldwide through environmental (contaminated drinking water, air, food and domestic fuel sources) and occupational exposures (smelting industries, pesticide production). Despite the recent decrease in the allowable U.S. maximum contaminant level of arsenic in drinking water from 50 to 10 ppb, excessive exposure continues to occur in the U.S. through contaminated well water as well as other non-regulated sources. In Arizona, 39% of the wells monitored have had arsenic levels above 10 ppb. Even in areas with low water arsenic concentrations, the high variability of urinary arsenic concentrations suggests that chronic exposure to arsenic remains a significant public health issue. Globally, environmental arsenic exposure continues to be of great concern.

At high concentrations, environmental arsenic exposure is associated with cancer, chronic obstructive pulmonary disease (COPD) and other respiratory illness. However, the toxicologic mechanisms for these findings have not been identified, and studies that directly evaluate the extent of respiratory effects from low (≤ 50 ppb) arsenic exposure are limited. At the University of Arizona we have formed a unique partnership among scientists studying the effects of arsenic on a cellular level (Dr. Scott Boitano), in animals (Dr. Clark Lantz) and in humans (Dr. Jeff Burgess).

In this presentation, we have chosen to focus on the effects of arsenic on airway remodeling. While remodeling occurs to some extent in healthy lungs, abnormal airway remodeling is an important step in the development of chronic lung disease. If the ratio of proteinases that break down the lung over antiproteinases that prevent this effect becomes unbalanced, the net result will be a loss of lung tissue or the development of abnormal lung structure. In addition, increased lung inflammation is associated with development of lung disease. Exploration of these mechanisms of injury due to arsenic is based on our research data demonstrating:

- In airway epithelial cell culture, dose-dependent increases in matrix metalloproteinase 9 (MMP-9) and MMP-9/tissue inhibitor of metalloproteinase 1 (TIMP-1) ratio, alteration of tight junctions between cells and decreased Ca^{2+} signaling associated with a reduction in wound healing;
- In mice, increased lung MMP-9 gene expression and protein levels and decreased receptor for advanced glycation end products (RAGE), the soluble form of which helps protect against the induction of inflammatory effects in the lung;
- In humans, a positive association of urine arsenic with sputum protease/antiprotease (MMP-9/TIMP-1) ratio and a negative association with sputum RAGE.

We have used these preliminary data to formulate a hypothetical mechanism for the development of respiratory disease such as COPD from low-level arsenic exposure (Figure 1).

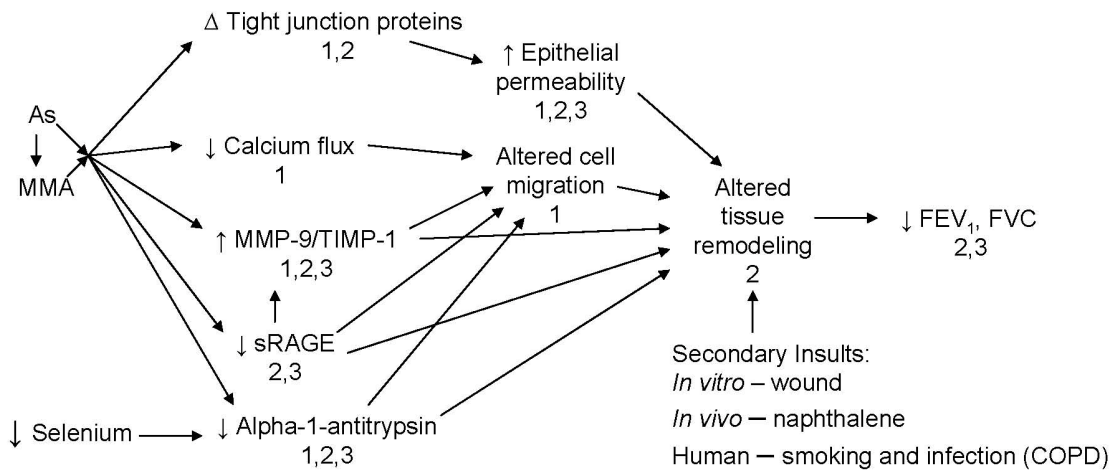


Figure 1. Postulated mechanisms of arsenic toxicity (1=Aim 1 (*In-vitro*); 2=Aim 2 (*In-vivo*); 3=Aim 3 (Human))

During the meeting I will present the results of our research studies to date involving arsenic exposure and respiratory effects, and discuss the ongoing research in this area. This and additional research involving effects in other parts of the body will be necessary to determine if the current 10 ppb for arsenic in drinking water is adequately protective against deleterious effects in exposed populations.

Preliminary Results of Metals Found in Drinking Water among Arizonan and Sonoran Residents.

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Environmental arsenic exists in several oxidative and physical states, with human exposure occurring primarily through ingestion or inhalation. A major source of inorganic arsenic exposure occurs via drinking water. In general, igneous intrusions in the western U.S. and the Sierra Madre Occidental are associated with increased groundwater arsenic concentrations, the major water source in our semi-arid study area. Arsenic exposure may be a causal agent in the development of kidney, bladder, lung and skin cancers.

The overall goal of this project is to evaluate the associations between arsenic exposure and selected genetic polymorphisms considered part of carcinogenesis. We have assembled a binational team of investigators from the University of Arizona and from two institutions in Mexico (Universidad de Sonora in Hermosillo and Instituto de Tecnologica de Sonora in Ciudad Obregon).

In Arizona, 225 people from 152 households were randomly selected to participate in this study from Ajo, New River, San Manuel, and Tucson. More than 200 households were selected to participate in Mexico. All participants were asked to provide a first morning urine void, DNA, serum, and toenail clipping samples in addition to completing a panel of questionnaires which capture demographic, health, diet and other exposure related data. Water samples were collected from every drinking water source in each of the households which included tap water (filtered and unfiltered) and bottled water.

The methods used for the analysis of these samples are two-fold: a CDC method to test trace metals in urine and a modified water method to test for metals in water. This trace metals screen detects the presence and concentration of antimony, arsenic, barium, beryllium, cadmium, cesium, cobalt, lead, molybdenum, platinum, selenium, thallium, tungsten, and uranium. An inductively coupled plasma – mass spectrometer (ICP-MS), more specifically a Perkin-Elmer ELAN DRC II was used to analyze the samples. This instrument has an optional hyphenated high pressure liquid chromatography front end that allows for the speciation of arsenic. The water sample preparation involved acidification of the samples using clean concentrated nitric acid and allowing the samples to sit for a minimum of 24 hours prior to analysis.

Sampling in Arizona and Ciudad Obregon is completed. Sampling in Hermosillo is near completion. In Arizona, 56% of the participants were female and 44% were male. 25% of the participants were Hispanic and 75% were Non-Hispanic. The arsenic levels from an unfiltered water source varied by city in Arizona: Ajo (3.9 – 156.9 ppb), New River (<3.5 – 19.6 ppb), San Manuel (<3.5 – 1094.3 ppb), and Tucson (<3.5 – 54.8 ppb).

Communities sampled in Ciudad Obregon were chosen based on the arsenic concentrations from 73 wells which are the main source of drinking water in the rural area of the Yaqui, Mayo and Guaymas Valleys, in Sonora, Mexico. Total arsenic concentration in the wells of the Guaymas Valley ranged between 8.41 to 10.16 ppb; in the Yaqui Valley between 0.73 to 45.23 ppb, and in the Navojoa Valley between 7.16 to 26.62 ppb. Over 58.33% of the well water samples exceeded the US-EPA standard of 10 ppb.

We report here our study design, progress to date and preliminary results.
SPORE in GI Cancer (NIH/NCI CA95060)

Regulatory Perspective from Arizona Department of Environmental Quality

Linda Taunt, Deputy Director of the Water Quality Division

The Arizona Department of Environmental Quality will discuss the regulatory side of the emerging contaminants issue. As the number of unregulated and emerging contaminants increase and the ability to detect the compounds improved, when is the right time for regulatory agencies to develop standards? How are standards actually developed?